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Indole in DNA: Comparison of a Nucleosidic with a Non-Nucleosidic DNA Base Substitution

Janez Barbaric,[a] Claudia Wanninger-Weiß,[a] and Hans-Achim Wagenknecht*[a]

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The synthetic incorporation of indole as an artificial DNA base into oligonucleotides by two different structural approaches is described. For both types of modification, the indole moiety is attached through the C-3 position to the oligonucleotides. As a mimic of natural nucleosides, the indole nucleoside of β-2′-deoxyribofuranoside (In) was synthesized. The corresponding In-modified duplexes were compared with duplexes that contained the indole group connected through (*S*)-3-amino-1,2-propanediol as an acyclic linker between the phosphodiester bridges of the oligonucleotides. This linker was tethered to the C-3 position of the indole heterocycle either directly (In′′) or by a carbamate function (In′). The melting temperatures of the corresponding indole-modified DNA duplexes were measured and compared. Interestingly, not only the In′ and In′′ modifications but also the natural-like In base surrogate destabilize the DNA duplex strongly. This result supports our approach to apply the acyclic glycol linker to incorporate aromatic molecules as artificial DNA base substitutions. The major advantage of acyclic glycol linkers [such as the applied (*S*)-3-amino-1,2-propanediol] is that the corresponding modifications are synthetically more easily and readily accessible, as it avoids the preparation of the nucleosidic bond and the separation and purification of the α- and β-anomers.

Introduction

The DNA helix is a supramolecular assembly that consists of a π-stacked one-dimensional array of four aromatic heterocycles that are attached as nucleosides to 2′-deoxyribofuranosides and held in place by two antiparallel phosphodiester backbones. The four natural DNA bases adenine, cytosine, guanine and thymine vary only slightly in polarity or stacking ability in comparison to the structural diversity of organic molecules that potentially could be incorporated into oligonucleotides as substitutions or surrogates for DNA bases. Simple aromatic hydrocarbons and aromatic heterocycles were among the first replacements for DNA bases without hydrogen-bonding capabilities.[1–4] Universal base analogues are molecules that can pair equally well with all four natural DNA bases,[1–4] like, for instance, 5-nitroindole.[5] As a result of the structural similarity with the two natural purine bases, the unsubstituted indole represents an important artificial DNA base that isosterically substitutes guanine or adenine in DNA.[6–16] Indole-containing nucleosides and oligonucleotides are also target molecules for bioanalytical and medicinal applications.[6–16]

Beside the significance for chemical biology and bioorganic chemistry, indole represents a very promising charge trap for charge transport studies in DNA owing to its low oxidation potential and the characteristic transient absorption of the corresponding radical or radical cation. Those charge transfer experiments have been performed with indole either as part of tryptophan in short DNA-binding peptides, mainly Lys-Trp-Lys,[17,18] or DNA-binding proteins.[19] Experiments with indole connected through the N-1 position as an artificial DNA base[20,21] revealed that the aromatic N–H group of indole is crucial for an efficient trapping of positive charges. The indole radical cation is very acidic ($pK_a \approx 4$) and couples the charge transfer with a deprotonation step.[22] Hence, the C-nucleoside with indole attached either by the C-3 or the C-2 position of the heterocycle is more desirable for charge transfer studies than the corresponding N-1 nucleoside.

Excluding the benzene part of indole, there are in principle three alternative positions for the attachment of the heterocycle to DNA: (i) The synthesis of the β-nucleoside of indole attached through the nitrogen in the 1-position to the anomeric centre of 2′-deoxyribofuranoside or ribofuranoside has been published several times.[6–9] The resulting artificial DNA base as part of oligonucleotides or nucleotide triphosphates has been used in DNA base pairing and stacking experiments and to probe DNA polymerase-catalyzed primer extension.[9–11] (ii) The synthesis of the C-nucleoside bearing the indole heterocycle attached through its C-2 position is found rarely in the literature.[12–14] This nucleoside has been used mainly as an unnatural base pair bearing the indole N–H group as a specific minor-groove

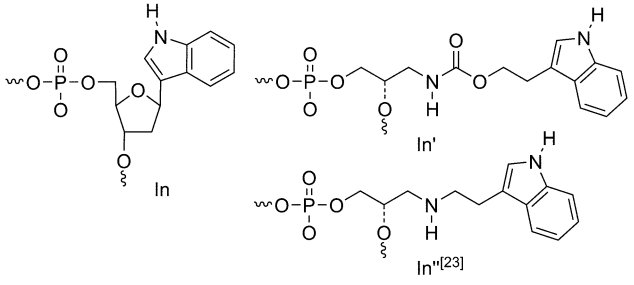
[a] Institute for Organic Chemistry, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany
Fax: +49-941-943-4617
E-mail: achim.wagenknecht@chemie.uni-regensburg.de

hydrogen-bond donor.[12] (iii) The synthesis of the C-nucleoside attached through the C-3 position of the indole heterocycle is known, as this position exhibits the highest reactivity towards electrophilic aromatic substitution.[8,15,16] However, the resulting indole C-nucleoside has not yet been incorporated into oligonucleotides.

Recently, we chose a different approach in order to study the stacking interactions of indole by incorporation as an artificial DNA base at specific sites in duplex DNA.[23] The 2′-deoxyribofuranoside moiety was replaced by (*S*)-3-amino-1,2-propanediol as an acyclic linker that is tethered to the C-3 position of the indole heterocycle. We used a similar synthetic approach for the incorporation of ethidium,[24] perylene bisimide,[25] phenothiazine[26] and thiazole orange[27] as DNA base surrogates. Avoiding the labile glycosidic bond, this approach provides the necessary chemical stability for the preparation of DNA modifications by automated phosphoramidite chemistry.[28] Similar propanediol linkers have been used by others to prepare glycol nucleic acid (GNA),[29] twisted intercalating nucleic acids (TINA)[30] and alkyne-modified oligonucleotides for the click-Huisgen cycloaddition as a postsynthetic modification.[31]

Results and Discussion

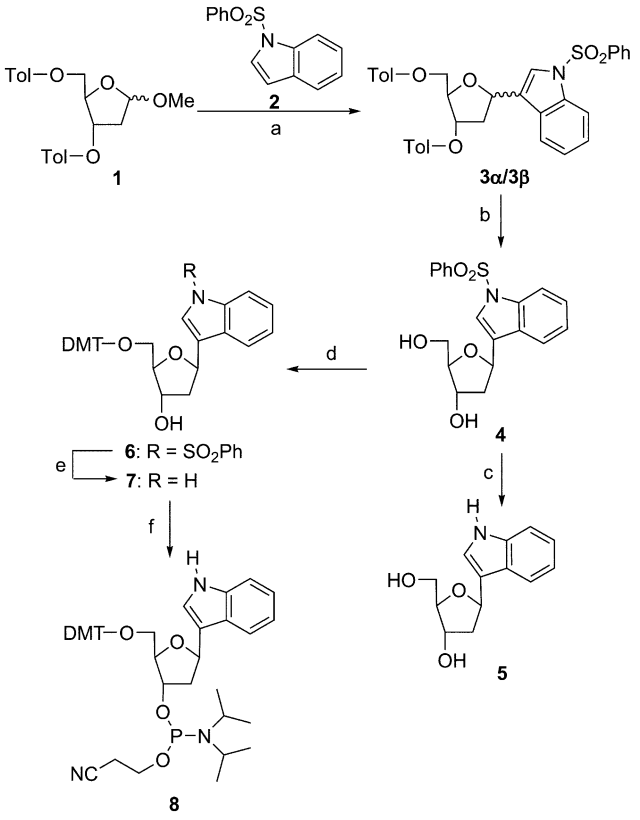
Herein, we present the synthetic routes for two different structural approaches to incorporate indole as an artificial DNA base into oligonucleotides through attachment to the C-3 position of the heterocycle. The natural-like C-nucleoside (In) containing the indole moiety tethered to the β-2′-deoxyfuranoside is compared with the non-nucleosidic alternative bearing (*S*)-3-amino-1,2-propanediol as an acyclic linker between the phosphodiester bridges (In′) (Scheme 1). In contrast to our previously published In′′,[23] the indole group of In′ is tethered to (*S*)-3-amino-1,2-propanediol through a carbamate function that facilitates the synthesis of the corresponding DNA building block, because protection of the N–H group of the linker part is no longer necessary (Scheme 1).



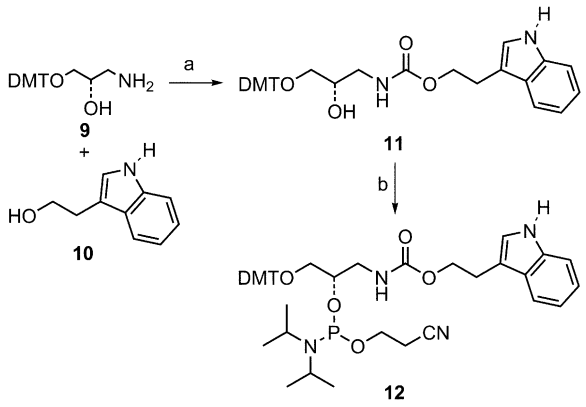
Scheme 1. Structural variations to incorporate indole as an artificial base into oligonucleotides.

The precursor for the synthesis of In-DNA building block **8** is 1-*O*-methyl-3,5-di-*O*-toluoyl-2-desoxyribose (**1**), which can be synthesized according to the literature (Scheme 2).[33] The key step of the synthetic route to **8** represents the C–C bond formation between the anomeric centre of **1** and the C-3 position of 1-phenylsulfonylindole (**2**). This reaction was performed through Lewis acid promoted electrophilic substitution at –15 °C. In this case, the Lewis acid $BF_3{\cdot}OEt_2$ catalyzes the formation[16] of *para*-toluoyl-protected C-nucleoside **3α/3β** in 35% isolated yield. The anomeric mixture **3α/3β** was separated by flash chromatography to afford pure **3β** in 26% yield and pure **3α** in 9% yield. The assignment of the two anomers is based on ^{1}H NMR spectroscopy. The multiplicity of the H-1′ signal (**3β**) shows a doublet of doublets (J = 5.2 Hz, J = 10.4 Hz) at δ = 5.43 ppm and is typical for the β-isomer.[1] The NOE studies of compound **3β** show interactions between H-2 and H-4 of the indole moiety and H-1′ and H-2α′. Thus, these contacts do not provide clear evidence for the β-anomer. However, the strong NOE contacts between H-1′ and H-2α ′ and H-1′ and H-4′ in the sugar part could only be explained by the existence of H-1α′ that is part of β-anomer **3β**. The stereoselectivity of the C–C bond formation depends on the temperature. Because **3β** is the thermodynamically controlled reaction product, high temperatures yield more β-product (at –15 °C **3β/3α** = 4:1). The deprotection of the 3′- and 5′-OH functions of **3β** was carried out by standard treatment with NaOMe in dry MeOH[32] to give **4** in 83% yield. Nucleoside **4** was deprotected with KOH in the presence of 18-crown-6 to the completely unprotected indole nucleoside **5**. The low yield of the last step was due to the decomposition of the product that was observed under the strongly basic conditions. Furthermore, the following tritylation of the 5′-OH-function of **5** could not be carried out in high yields. Hence, for the later synthetic incorporation into oligonucleotides it turned out to be useful to first introduce the DMT-protecting group to the 5′-OH function of **4** and subsequently desulfonylate the NH function of **6**. By using this synthetic route, **7** could be obtained in 55% yield for both steps. The synthesis of In-phosphoramidite **8** was finished by using standard procedures.[23]

For the non-nucleosidic In′ and In′′ modifications the *S* configuration of the acyclic linker was chosen to mimic the stereochemical situation at the 3′-position of natural 2′-deoxyribofuranosides. We showed recently that the synthesis of the In′′-DNA building block could be accomplished in a three-step route.[23] However, the secondary amine function of the linker system in the In′′-type conjugate needs to be protected with a trifluoroacetyl group to avoid irreversible acetylation at this position during the capping steps of the DNA synthesizer cycle. Especially, this reaction turned out to be a real bottleneck of the synthetic route, as it was difficult to remove the trifluoroacetate from the hydroxy function of the linker molecule while keeping it at the amine functionality. To prevent this protection step and to therefore simplify the synthesis we chose a carbamate function to link the indole moiety and the acyclic linker for In′-DNA building block **12** (Scheme 3). Because of the low basicity of the NH of the carbamate group, protection is not necessary during the oligonucleotide synthesis. DMT-protected (*S*)-3-amino-1,2-propanediol **9** as the precursor was synthesized according to the literature.[24] The hydroxy function of com-



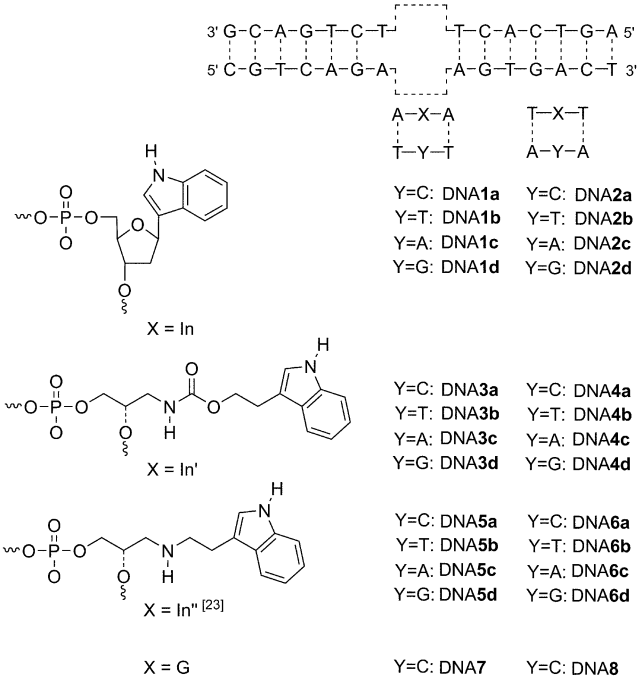
Scheme 2. Synthesis of In-phosphoramidite **8**. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$ (6.9 equiv.), CH_2Cl_2, –15 °C, 1.5 h, 9% (**3α**), 26% (**3β**); (b) NaOMe (3 equiv.), MeOH, 6 h, room temp., 83%; (c) 18-crown-6 (1.5 equiv.), KOH (33 equiv.), MeOH, 1,4-dioxane, 2 h, room temp., 15%; (d) DMTCl (1.3 equiv.), NEt_3 (3 equiv.), pyridine, room temp., 16 h, 69%; (e) 18-crown-6 (1.5 equiv.), KOH (33 equiv.), MeOH, 1,4-dioxane, room temp., 16 h, 80%; (f) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1.5 equiv.), NEt_3 (3 equiv.), CH_2Cl_2, room temp., 45 min, 95%.



Scheme 3. Synthesis of In′-phosphoramidite **12**. Reagents and conditions: (a) CDI (1.1 equiv.), DMF, room temp., 3 d, 69%; (b) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (1 equiv.), $EtN(iPr)_2$ (3.6. equiv.), CH_2Cl_2, room temp., 30 min, 96%.

mercially available 3-(2-hydroxyethyl)indole (**10**) was converted into an activated ester by 1,1′-carbonyldiimidazole and subsequent nucleophilic acyl substitution with **9** gave conjugate **11** in 69% yield. The synthesis of phosphoramidite **12** was accomplished by standard procedures.

All In- and In′-modified oligonucleotides presented in this manuscript were prepared by automated solid-phase synthesis on an Expedite 8909 DNA synthesizer. Near-quantitative coupling of the indole building blocks was achieved only when the coupling time was extended to 15 min for phosporamidite **8** and 30 min for phosphoramidite **12**. The HPLC-purified oligonucleotides were identified by MS (ESI) and quantified by their UV/Vis absorption by using ε_{260} = 3900 $M^{-1}cm^{-1}$ (In) and ε_{260} = 4000 $M^{-1}cm^{-1}$ (In′). We prepared two sets of duplexes for each modification that contained two sequential variations to study the In and In′ modifications (Scheme 4): (i) The bases directly adjacent on each side of the modification site were either A (DNA**1**/DNA**3**) or T (DNA**2**/DNA**4**), as representatives for purines and pyrimidines. (ii) The counterbase opposite to the In or In′ modification was either C, T, A or G (DNA**Xa**–DNA**Xd**). Additionally, we compared the In-modified and In′-modified duplexes with the previously published In′′-modified ones (DNA**5**, DNA**6**) and the completely unmodified duplexes DNA**7** and DNA**8** bearing a G instead of any indole-containing artificial base pair.



Scheme 4. Sequences of In-modified DNA**1a**–DNA**2d**, In′-modified DNA**3a**–DNA**4d**, In′′-modified DNA**5a**–DNA**6d**[23] and unmodified duplexes DNA**7**[23] and DNA**8**.[23]

To study the stacking situation and to evaluate the influence of the indole moieties we determined the thermal stability of all modified duplexes by measuring the melting temperatures (T_m) at 260 nm and compared them with T_m values of the corresponding unmodified duplexes DNA**7** and DNA**8** (Table 1). Three major results can be drawn from this data: (i) The melting temperature of all modified duplexes lie in a rather narrow range of 51–55 °C. (ii) All

modified duplexes show a strong destabilization by 11–15 °C compared to the unmodified duplexes. (iii) None of the indole modifications exhibit any preferred pairing with one special counterbase in the complementary strand.

Table 1. Melting temperatures (T_m)[a] of In-modified DNA**1a**–DNA**2d**, In′-modified DNA**3a**–DNA**4d**, In′′-modified DNA**5a**–DNA**6d**[23] and unmodified duplexes DNA**7**[23] and DNA**8**.[23]

X	Y			
	C Duplex T_m [°C]	T Duplex T_m [°C]	A Duplex T_m [°C]	G Duplex T_m [°C]
In	DNA**1a** 51.0 DNA**2a** 52.0	DNA**1b** 53.0 DNA**2b** 52.5	DNA**1c** 54.0 DNA**2c** 53.0	DNA**1d** 54.0 DNA**2d** 51.8
In′	DNA**3a** 55.0 DNA**4a** 53.0	DNA**3b** 54.7 DNA**4b** 53.2	DNA**3c** 54.5 DNA**4c** 53.2	DNA**3d** 54.5 DNA**4d** 52.7
In′′	DNA**5a**[23] 52.0 DNA**6a**[23] 52.9	DNA**5b**[23] 54.3 DNA**6b**[23] 51.8	DNA**5c**[23] 54.2 DNA**6c**[23] 53.5	DNA**5d**[23] 53.8 DNA**6d**[23] 54.0
G	DNA**7**[23] 65.0 DNA**8** [23] 65.8			

[a] Conditions: λ = 260 nm, 10–90 °C, interval: 0.7 °C min^{-1}, 2.5 μM duplex in 10 mM Na-P$_i$ buffer, 250 mM NaCl, pH 7.

For the In′ (DNA**3a**–DNA**4d**) and In′′ modification (DNA**5a**–DNA**6d**), the destabilizing effect is not surprising, as the glycol linker substitutes the 2′-deoxyribofuranoside. The major difference in this linker relative to the natural 2′-deoxyribofuranoside is the number of carbon atoms between the phosphodiester bridges, which has been reduced from three (in normal nucleosides) to two (in In′ and In′′). The studies of glycol nucleic acids (GNA) by Meggers and coworkers revealed also that a single glycol modification typically destabilizes the duplex significantly.[29] According to our experience, this depends on the size of the hydrophobic surface of the artificial DNA base surrogate if some of the lost duplex stability that is caused by the glycol linker can be regained by stacking interactions. For instance, DNA duplexes that have been modified by perylene bisimide chromophores attached through the (*S*)-3-amino-1,2-propanediol linker between the phosphodiester bridges showed no significant destabilization.[25] This result was attributed mainly to the hydrophobic interactions of those chromophores.

More surprising is the observation that the duplexes with the In modification (DNA**1a**–DNA**2d**) show a similarly strong destabilization relative to the duplexes with the In′ or In′′ modifications. The nucleosidic In modification with β-configuration at the anomeric centre resembles the structure of natural nucleosides best possible. It was designed as an isosterical surrogate for purine DNA bases. In the *anti* conformation of the In nucleosides, the NH group of the indole moiety probably points into the major groove. Hence, the destabilization effect of the In-modified duplexes has to be assigned to the lack of hydrogen-bonding interactions of the artificial DNA base In. This is an important observation, because it rules out that the glycol linker, which contains one carbon atom less between the phosphodiester bridges (relative to 2′-deoxyribofuranoside), is the single cause for destabilization of the duplex.

With respect to the potential applicability of the In base as a universal base analogue it is important to measure the influence of the counterbase to the indole modification site (In). In fact, all three indole modifications (In, In′ and In′′) do not exhibit any preference in base-pairing selectivity according to the melting temperatures. Hence, they all can be considered as universal base analogues. For the duplexes DNA**Xa** and DNA**Xb** this result was expected, because indole represents an isosteric substitution for purines. However, for the duplexes DNA**Xc** and DNA**Xb** bearing purines as counterbases a further destabilization was expected.

Conclusions

We showed the syntheses of two structurally different incorporations of indole as an artificial DNA base into oligonucleotides. For both types, the indole moiety was attached through the C-3 position to the oligonucleotides. As a mimic of natural nucleosides we used the indole nucleoside of β-2′-deoxyribofuranoside (In). The corresponding In-modified duplexes were compared with duplexes that connected the indole heterocycle with the use of (*S*)-3-amino-1,2-propanediol as an acyclic linker between the phosphodiester bridges of the oligonucleotides. This linker was tethered to indole either directly (In′′)[23] or through a carbamate function (In′). We measured and compared the melting temperatures of the corresponding modified DNA duplexes. The acyclic indole modifications In′ and In′′ strongly decrease the duplex stability. Surprisingly, the same effect occurs by using the natural-like In nucleoside. According to these results it is obvious that the destabilizing effect does not depend on the modality of the chromophore attachment to the oligonucleotide. Consequently, in the future the acyclic linker can preferably be chosen for the incorporation of artificial DNA bases, for example, fluorophores. This is advantageous, because the corresponding DNA building blocks are synthetically more easily and readily accessible, as the preparation of the nucleosidic bond and the separation and purification of the α- and β-anomers can be avoided.

Experimental Section

Materials and Methods: ^{1}H, ^{13}C, ^{31}P and 2D NMR spectra were recorded with a Bruker Avance 300 or Avance 400 NMR spectrometer. ESI and FAB mass spectra were measured in the analytical facility of the institute with a Finnigan TSQ 7000 or Finnigan MAT 95. HRMS (ESI) was measured by Coring System Diagnostix GmbH, Gernsheim. Analytical chromatography was performed

on Merck silica gel 60 F254 plates. Flash chromatography was performed on Merck silica gel (40–63 μm). Solvents were dried according to standard procedures. All reactions were carried out under a nitrogen atmosphere and protected from light. Reagents and chemicals were obtained from Alfa Aesar, Fluka and Lancaster and were used without further purification. The oligonucleotides were prepared with an Expedite 8909 DNA synthesizer (ABI) by using CPG (1 μmol) and chemicals from ABI and Proligo. The trityl-off oligonucleotides were cleaved and deprotected by treatment with concentrated NH_4OH at 60 °C for 10 h, dried and purified by HPLC by using the following conditions: A = NH_4OAc buffer (50 mM), pH 6.5; B = MeCN; gradient 0–20% over 70 min for In-modified oligonucleotides and 5–15% over 55 min of In′-modified oligonucleotides. C18-RP analytical and semipreparative HPLC columns (300 Å) were purchased from Supelco. Duplexes were formed by heating to 90 °C (10 min), followed by slow cooling. Melting temperature measurements were performed with a Cary 100 (Varian) with duplex samples (2.5 μM) in quartz glass cuvettes (1 cm) and in Na-P_i buffer (10 mM), NaCl (250 mM), pH 7, 260 nm, interval 0.7 °C min^{-1}.

1-Phenylsulfonyl-3-(2′-deoxy-3′,5′-di-*O*-para-toluoyl-β-ribofuranosyl)indole (3α/3β): To a solution of 1-*O*-methyl-3,5-di-*O*-toluoyl-2-desoxyribose (**1**; 6.18 g, 16.09 mmol) and 1-phenylsulfonylindole (**2**; 5.1 g, 19.32 mmol) in dry CH_2Cl_2 (15 mL) was added $BF_3{\cdot}OEt_2$ (14 mL, 35.9 mmol) at –15 °C. After stirring for 1.5 h at –15 °C, the reaction mixture was treated with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel; hexanes/EtOAc, 8:1) to yield **3α** (0.83 g, 8.5%) and **3β** (2.58 g, 26%) as white foams. Data for **3α**: R_f = 0.41 (hexanes/EtOAc, 3:1). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 7.94–7.88 (m, 6 H, Ar-H), 7.60 (m, 2 H, Ar-H), 7.50 (m, 1 H, Ar-H), 7.39 (m, 2 H, Ar-H), 7.31–7.21 (m, 4 H, Ar-H), 7.06 (m, 1 H, Ar-H), 5.61 (m, 1 H, H3′), 5.55 (t, *J* = 5.8 Hz, 1 H, H1′), 4.56 (m, 2 H, H5′), 4.53 (m, *J* = 4.5, *J* = 11.8 Hz, 1 H, H5′), 4.51 (m, 1 H, H4′), 2.99 (m, H2′β), 2.50 (m, 1 H, H2′α), 2.39 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (75.5 MHz, [D_6]-DMSO): δ = 166.7, 166.4, 144.2, 144.1, 137.8, 133.9, 129.45, 129.4, 129.1, 129.0, 128.8, 126.83, 126.6, 126.5, 124.7, 123.6, 123.2, 123.1, 120.2, 111.4, 82.1, 78.1, 77.7, 77.3, 76.5, 74.3, 64.2, 37.6, 20.6 ppm. HRMS (ESI): calcd for $C_{35}H_{31}NO_7S$ 609.1821 [M + H]$^+$ found 610.1890. Data for **3β**: R_f = 0.44 (hexanes/EtOAc, 3:1). ^{1}H NMR (600 MHz, [D_6]DMSO): δ = 7.96–7.91 (m, 4 H, *p*-Tol, H2 and H6), 7.96–7.91 (m, 2 H, SO_2-Ph, H2 and H6), 7.83 (s, 1 H, Ar_{Ind}-H2), 7.69–61 (m, 1 H, Ar_{Ind}-H6), 7.51 (t, *J* = 8.3 Hz, 1 H, Ar_{Ind}-H5), 7.34–7.27 (m, 4 H, *p*-Tol, H3 and H5), 7.34–7.27 (m, 2 H, SO_2-Ph, H3 and H5), 7.06–7.03 (t, *J* = 7.4 Hz, 1 H, SO_2-Ph, H4), 5.61 (d, *J* = 5.8 Hz, 1 H, H3′), 5.43 (dd, *J* = 5.2, 10.4 Hz, 1 H, H1′), 4.61 (dd, *J* = 4, 11.8 Hz, 1 H, H5′), 4.53 (dd, *J* = 4.5, 11.8 Hz, 1 H, H5′), 4.46 (m, 1 H, H4′), 2.55 (dd, *J* = 5.5, 13.3 Hz, 1 H, H2′α), 2.46 (m, 1 H, H2′β), 2.39 (s, 3 H, CH_3), 2.36 (s, 3 H, CH_3) ppm. ^{13}C NMR (151 MHz, [D_6]DMSO): δ = 165.5, 165.4, 143.9, 143.8, 136.9, 134.7, 134.6, 129.8, 129.4, 129.3, 129.3, 129.2, 128.4, 126.7, 126.7, 126.6, 125.0, 123.7, 123.1, 122.2, 120.9, 113.2, 82.1, 76.6, 73.8, 64.3, 21.2, 21.1 ppm. HRMS (ESI): calcd. for $C_{35}H_{31}NO_7S$ 609.1821 [M + H]$^+$; found 610.1890.

1-Phenylsulfonyl-3-(2′-deoxy-β-D-ribofuranosyl)indole (4): NaOMe (183 mg, 3.39 mmol) was added to a solution of **3β** (692 mg, 1.13 mmol) in dry MeOH (20 mL). After 6 h stirring at room temperature, the solution was evaporated to dryness. The residue was purified by flash chromatography (silica gel; toluene/CH_2Cl_2/MeOH, 5:5:1 to 1:1:1) to yield **4** (350 mg, 83%) as a yellow powder. R_f = 0.25 (toluene/$CHCl_2$/MeOH, 5:5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99–7.97 (d, *J* = 8.2 Hz, 1 H, Ar_{Ind}-H, H7), 7.97–7.87 (m, 2 H, SO_2-Ph, 2-H and H6), 7.6–7.57 (d, *J* = 7.8 Hz, Ar_{Ind}-H, H4), 7.56–7.51 (m, 1 H, SO_2-Ph, H3), 7.53 (s, Ar_{Ind}-H, H2), 7.46–7.41 (m, 2 H, SO_2-Ph, H2 and H4), 7.36–7.30 (dt, *J* = 1.7, 7.2 Hz, 1 H, Ar_{Ind}-H, H6), 7.26–7.21 (dt, *J* = 0.8, 7.5 Hz, 1 H, Ar_{Ind}-H, H5), 5.36 (dd, *J* = 9, 6 Hz, 1 H, H1′), 4.5 (m, 1 H, H3′), 4.02 (dt, *J* = 3.4, 4.3 Hz, 1 H, H4′), 3.78 (m, 2 H, H5′), 2.3–2.23 (m, 2 H, H1′α, H1′β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.5, 133.8, 130.1, 129.3, 128.9, 126.8, 125.0, 123.4, 122.9, 122.6, 120.3, 113.7, 87.1, 73.7, 73.6, 63.1, 41.8, 30.9, 29.7 ppm. MS (ESI): *m*/*z* (%) = 374.1 (100) [M + H]$^+$, 391.2 (90) [M + NH_4]$^+$.

3-(2-Deoxy-β-D-ribosyl)indole (5): A mixture of **4** (1.0 g, 1.65 mmol), 18-crown-6 (646 mg, 2.47 mmol), KOH (2.0 g, 35.8 mmol), dry MeOH (5 mL) and dry 1,4-dioxane (5 mL) was stirred for 2 h at room temperature. The resulting mixture was extracted with CH_2Cl_2, dried with Na_2SO_4 and concentrated under vacuum. Flash chromatography (Et_2O/EtOAc, 2:1 to 1:3) yielded **5** (60 mg, 15%). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 10.91 (br. s, 1 H, NH), 7.56 (d, *J* = 7.9 Hz, Ar-H, H7), 7.33 (d, *J* = 8.1 Hz, 1 H, Ar-H, H4), 7.22 (d, *J* = 2.2 Hz, Ar-H, H2), 7.08 (dt, *J* = 7.9, 1.1 Hz, 1 H, H6), 6.95 (dt, *J* = 7.9, 0.9 Hz, 1 H, H5), 4.94 (dd, *J* = 11.2, 1.6 Hz, 1 H, H1′), 4.0 (s, 1 H, H3′), 3.5 (m, 1 H, H4′), 3.49–3.39 (dq, *J* = 5, 17.7 Hz, 2 H, H5′), 2.15 (dd, *J* = 9.3, 19.5 Hz, 1 H, H2′α), 1.95 (m, 1 H, H2′β) ppm. ^{13}C NMR (151 MHz, [D_6]-DMSO): δ = 136.6, 125.9, 121.0, 119.5, 118.3, 116.4, 111.4, 72.2, 70.4, 68.6, 67.4, 66.3, 60.3 ppm. MS (ESI): *m*/*z* (%) = 234.1 (100) [M + H]$^+$, 251.1 (50) [M + NH_4]$^+$, 484.3 (30) [2M + NH_4]$^+$, 489.3 (25) [2M + Na]$^+$.

1-Phenylsulfonyl-3-[2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]indole (6): 4,4′-Dimethoxy-triphenylmethyl chloride (411 mg, 1.21 mmol) and dry Et_3N (392 μL, 2.79 mmol) was added to a solution of **4** (350 mg, 0.93 mmol) in dry pyridine (10 mL). The mixture was stirred overnight at room temperature. Subsequently, MeOH (5 mL) was added. After 1 h at room temperature, the solution was concentrated to dryness. The crude product was purified by flash chromatography (hexanes/EtOAc, 2:1 + 0.1% Et_3N) to yield **6** (440 mg, 69%) as a yellow oil. R_f = 0.5 (hexane/EtOAc, 1:1). ^{1}H NMR (300 MHz, [D_4]MeOD): δ = 7.95 (d, *J* = 8.5 Hz, 1 H, Ar_{Ind}-H, H7), 7.73–7.66 (m, 4 H, Ar-H), 7.44–7.40 (dd, 2 H, Ar-H), 7.32–7.25 (m, 10 H, Ar-H), 7.08–7.03 (t, 2 H, Ar-H), 6.70–6.60 (2dd, *J* = 6.9, 2.2 Hz, 4 H, 2 Ar_{DMT}-H, H3 and H5), 5.29 (t, *J* = 8.5 Hz, 1 H, H1′), 4.94 (d, *J* = 4 Hz, 1 H, H3′), 4.00 (br. s, 1 H, 3′-OH), 3.93 (m, 1 H, H4′), 3.69 (s, 3 H, OMe), 3.68 (s, 3 H, OMe) 3.12–3.03 (m, 2 H, H5′), 2.2 (m, 2 H, H2′α und H2′β) ppm. ^{13}C NMR (100 MHz, [D_4]MeOD): δ = 158.0, 157.9, 149.6, 144.9, 136.8, 136.0, 135.6, 135.5, 134.7, 134.5, 129.7, 129.6, 128.8, 127.7, 126.6, 126.5, 124.9, 124.0, 123.8, 123.2, 122.6, 121.1, 113.1, 113.1, 113.0, 85.9, 85.3, 73.1, 72.2, 64.3, 54.9, 41.3, 41.2, 29.5 ppm. MS (ESI): *m*/*z* (%) = 734.3 (100) [M + CH_3COO]$^-$, 710.2 (25) [M + Cl^-]$^-$.

3-[2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]indole (7): A mixture of compound **6** (440 mg, 0.65 mmol), 18-crown-6 (262.5 mg, 0.975 mmol), KOH (1.21 g, 21.5 mmol), dry MeOH (10 mL) and 1,4-dioxane (10 mL) was stirred overnight at room temperature. The resulting mixture was evaporated to dryness. Flash chromatography (hexane/EtOAc, 1:1) yielded **7** (320 mg, 80%) as a yellow oil. R_f = 0.41 (hexane/EtOAc, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (d, 1 H, Ar_{Ind}-H, H7), 7.62–7.58 (m, 2 H, Ar-H), 7.47–7.19 (m, 14 H, Ar-H), 7.07 (m, 2 H, Ar-H), 6.9–6.81 (m, 5 H, Ar-H), 5.29 (dd, *J* = 5.4, 10.2 Hz, 1 H, H1′), 4.54 (m, 1 H, H3′), 4.1 (m, 1 H, H4′), 3.75 (s, 3 H, OMe), 3.74 (s, 3 H, OMe) 3.12–3.03 (m, 2 H, H5′), 2.2 (m, 1 H, H2′α), 2.05 (m, 1 H,

H2′β) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.9, 157.7, 144.9, 136.8, 137.0, 135.7, 135.5, 134.5, 129.8, 129.6, 128.8, 127.6, 127.5, 127.3, 126.6, 124.3, 123.2, 113.0, 112.7, 85.2, 85.1, 73.0, 68.6, 64.1, 56.5, 54.92, 29.5 ppm. MS (ESI): *m*/*z* (%) = 594.4 (100) [M + $CH_3COO]^-$, 534.4 (28) [M – H]$^-$.

2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-1-(3-indolyl)-β-D-ribofuranose-3′-*O*-[(2-cyanoethyl)-*N*,*N*-diisopropyl]phosphoramidite (8): To a solution of **7** (160 mg, 0.299 mmol) dissolved in dry CH_2Cl_2 (10 mL) was added Et_3N (126.5 μL, 0.9 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (106 μL, 0.45 mmol), and the solution was stirred for 1 h at room temperature. The reaction was quenched by pouring it into aqueous saturated $NaHCO_3$ (20 mL), and the solution was then extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated to yield phosphoramidite **8** as a yellow foam, which was used directly for the oligonucleotide synthesis. R_f = 0.85 (hexane/EtOAc, 2:1). ^{31}P NMR (121.5 MHz, $CDCl_3$): δ = 148.8, 147.9 ppm.

2-(1*H*-Indol-3-yl)ethyl {(*S*)-3-[Bis(4-methoxyphenyl)phenylmethoxy]-2-hydroxypropyl}carbamate (11): 3-(2-Hydroxyethyl)indole (**10**; 80.5 mg, 0.50 mmol) and 1,1′-carbonyldiimidazole (89.2 mg, 0.55 mmol) were dissolved in dry DMF (8 mL) and stirred for 2 h at room temperature. Compound **9**[24] (196.5 mg, 0.50 mmol) was then added. The reaction mixture was stirred at room temperature for 3 d. The solvent was removed under vacuum. The crude product was purified by flash chromatography (EtOAc/CH_2Cl_2, 1:1 + 0.4% NEt_3) to yield **11** (200 mg, 69%) as a colourless solid. R_f = 0.65 (EtOAc/CH_2Cl_2, 10:3). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.82 (br. s, 1 H, NH), 7.53 (d, 1 H, Ar_{Ind}-H2), 7.39 (psd, 2 H, Ar_{Ind}-H), 7.34–7.19 (m, 9 H, Ar-H), 7.05 (pst, 1 H, Ar_{Ind}-H), 6.79 (pst, 1 H, Ar_{Ind}-H), 6.86 (m, 4 H, Ar-H), 4.88 (d, 1 H, OH), 4.14 (t, 2 H, CH_2-indole), 3.71 (m, 7 H, 2 OCH_3 and *H*-COH), 3.15 (m, 1 H, COO-CH), 2.92 [m, 5 H, COO-CH, 2-CH_2 (linker)] ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 158.0, 156.5, 145.1, 136.12, 135.8, 135.8, 129.7 (Ar-H), 127.7 (Ar-H), 127.7 (Ar-H), 127.1 (Ar_{Ind}-H), 126.5, 123.0 (Ar-H), 120.9 (Ar_{Ind}-H), 118.3 (Ar_{Ind}-H), 118.2 (Ar_{Ind}-H2), 113.0 (Ar-H), 111.3 (Ar-H), 110.2, 85.14, 68.8, 65.8, 63.9, 55.0 (OCH_3), 44.3, 24.9 ppm. MS (ESI): *m*/*z* (%) = 579.4 (100) [M – H]$^-$, 615.4 (13) [M + Cl]$^-$, 639.5 (35) [M + $CH_3COO]^-$. HRMS (FAB): calcd. for $C_{35}H_{35}N_2O_6$ 579.2495 [M – H]$^-$ found 579.2499.

2-(1*H*-Indol-3-yl)ethyl {(*S*)-3-[Bis(4-methoxyphenyl)phenylmethoxy]-2-[(2-cyanoethoxy)diisopropylaminophosphanyloxy]propyl}-carbamate (12): To a solution of **11** (104.4 mg, 0.18 mmol) dissolved in dry CH_2Cl_2 (6 mL) was added dry EtN(*i*Pr)$_2$ (90 μL, 0.648 mmol) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (40.3 μL, 0.18 mmol), and the mixture was stirred at room temperature for 30 min. The reaction was quenched with dry EtOH (100 μL) and quickly washed with freshly prepared aqueous $NaHCO_3$ solution. The organic phase was dried with Na_2SO_4, and the solvent was removed under vacuum to yield **12** (135 mg, 96%) as a pale brown, viscous liquid. R_f = 0.60 (CH_2Cl_2/MeOH, 100:3). ^{31}P NMR (121.5 MHz, [D$_6$]DMSO): δ = 149.7, 149.1 ppm.

General Procedure for the Solid-phase Synthesis of the In- and In′-Modified Oligonucleotides: The synthesis were performed on a 1 μmol scale (CPG 500 Å, Proligo) by using standard phosphoramidite protocols. Quantitative coupling of **8** was achieved by using a coupling time of 15 min. The coupling time of **14** was 30 min. After preparation, the oligonucleotides were quantified by their absorbance at 260 nm and by using ε (In) = 3900 $M^{-1}cm^{-1}$, ε (In′) = 4000 $M^{-1}cm^{-1}$. Data for ss-DNA**1**: MS (ESI–): *m*/*z* = 1289.3 [M – 4H]$^{4-}$. Data for ss-DNA**2**: MS (ESI–): *m*/*z* = 1284.6 [M – 4H]$^{4-}$. Data for ss-DNA**3**: MS (ESI–): *m*/*z* = 1300.4 [M – 4H]$^{4-}$, 1734.1 [M – 3H]$^{3-}$. Data for ss-DNA**4**: MS (ESI–): *m*/*z* = 1297.9 [M – 4H]$^{4-}$, 1730.1 [M – 3H]$^{3-}$.

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