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# A Conformationally Locked Aminomethyl *C*-Glycoside and Studies on Its *N*-Pyren-1-ylcarbonyl Derivative Inserted into Oligodeoxynucleotides

**Carlo Verhagen,[a] Torsten Bryld,[a] Michael Raunkjær,[a] Stefan Vogel,[a] Katerina Buchalová,[a] and Jesper Wengel*[a]**

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A new conformationally locked aminomethyl *C*-glycoside has been synthesized and incorporated into oligonucleotides (ONs). The applicability of the monomer in *post*-ON synthesis (i.e., conjugation by organic synthesis performed on ONs attached to resin) has been successfully demonstrated by condensation with pyren-1-ylcarboxylic acid. The abilities of the pyrenyl-derivatized ONs to recognize abasic sites in complementary strands were investigated by thermal denaturation studies, which showed the obtained pyrenyl-derivatized ONs to be promising candidates for this purpose. The synthetic route also allowed synthesis of a conformationally locked dipeptide mimetic suitable for use in peptide and carbohydrate chemistry.



## Introduction

A variety of modified oligonucleotides (ONs) have been synthesized in attempts to obtain antisense molecules with significantly improved pharmaceutical properties. The introduction of Locked Nucleic Acid (LNA)[1–3] has contributed to this development through its unprecedented ability to recognize complementary RNA targets. The constitution of LNA (i.e., the presence of the conformationally locked bicyclic sugar unit in place of the flexible ribose unit of the natural nucleotide-based backbone) is the factor that mediates this behavior. We now present further exploitation of the LNA scaffold through the introduction of the C-glycoside precursor **X** for use in conjugation chemistry on a *post-ON* synthesis level (Figure 1).[4] The advantage of such a methodology is the potential to synthesize a vast number of conjugates from only one precursor by means of peptide coupling chemistry. We have previously published the synthesis and hybridization properties of a monomer containing a pyrene moiety directly attached to a bicyclic locked skeleton (monomer **W**, Figure 1),[5] but putative modeling indicated an advantage in having the pyrene moiety located further away from the ribose into the core of the DNA:DNA duplex, resulting in enhancement of base stacking. Monomer **Z** (Figure 1) was designed to investigate this hypothesis, as the pyrene moiety here should have a higher degree of positional freedom due to the longer linker in monomer **Z** in relation to monomer **W**. The higher flexibility would presumably allow the pyrene moiety to orientate for optimal base stacking. ONs with incorporated monomer **Z** were subjected to thermal denaturation studies in order to evaluate the effect of the change in design relative to monomer **W**.

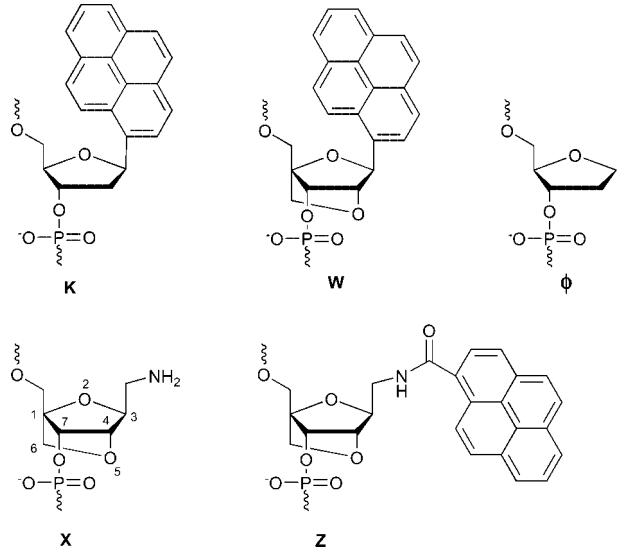


Figure 1. Chemical structures of monomers.

Here we further demonstrate the applicability of monomer **Z** to recognition of abasic sites (**Φ**, Figure 1). This capability has previously been demonstrated for ONs incorporating monomer **K**,[6–8] having a pyrene moiety directly substituting for a nucleobase. Monomer **K**[8] and monomer **W** have shown interesting universal base properties, and similar behavior is reported here for monomer **Z**.

[a] Nucleic Acid Center, Department of Chemistry, University of Southern Denmark,
5230 Odense M, Denmark
Fax: +45-66158780
E-mail: jwe@chem.sdu.dk

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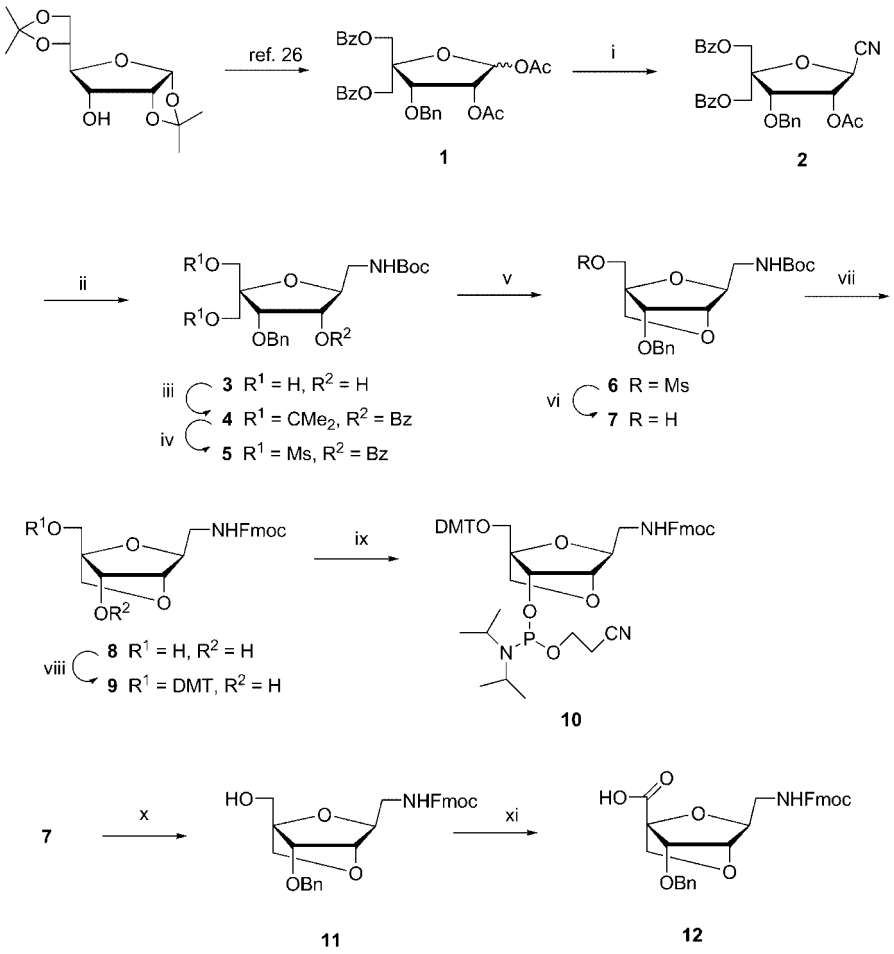


Scheme 1. i) TMSCN, $BF_3{\cdot}Et_2O$, $CH_3CN$, 68%. ii) a) $BH_3{\cdot}S(Me)_2$, THF, b) HCl, THF, c) NaOH, EtOH, d) $Boc_2O$, $CH_2Cl_2$, 56%. iii) a) 2,2-dimethoxypropane, TsOH, THF ($R^1 = R^1 = CMe_2$ indicates isopropylidene group), b) BzCl, TEA, $CH_2Cl_2$, 84%. iv) a) TsOH, MeOH, b) MsCl, TEA, 73%. v) NaOH, THF, 84%. vi) a) NaOBz, DMF, b) NaOH, EtOH, 73%. vii) a) $H_2$, 10% Pd/C, HCl, EtOH, b) FmocCl, MgO, 77%. viii) DMTCl, TEA, $CH_2Cl_2$, $CH_3CN$, 71%. ix) $(iPr)_2NP(Cl)OCH_2CH_2CN$, DIPEA, DCM, 57%. x) a) TFA, b) FmocCl, MgO, THF, 86%. xi) a) Dess–Martin periodinane, $CH_2Cl_2$, b) $NaH_2PO_4$, $NaClO_2$, *t*BuOH, 2-methylbut-2-ene, 76%.

Furthermore, only a few changes in the synthetic strategy enabled the synthesis of the novel, rigid LNA-based linker **12** (Scheme 1). The six-atom linker is suitable for use in solid-phase peptide synthesis, whilst the structural design of the building block contributes to the still expanding family of sugar amino acids (SAAs)[9–11] resembling conformationally restrained dipeptide isosters.[12–17] Members of this class of SAAs have found application in foldamers,[18,19] host-guest studies,[20] and as turn inducers.[21] We suggest SAA **12** as a promising candidate for such applications, due to the rigid nature of the molecule.

## Results and Discussion

The synthetic approach for construction of the bicyclic scaffold was based on the reported LNA synthesis methodology,[22] though the introduction of a novel aminomethylene functionality implied a change of strategy. We expected that the amino functionality should be readily accessible from a cyano-derivatized carbohydrate. Our choice of strategy involved Schmidt coupling chemistry[23–25] on the known carbohydrate **1**,[26] providing a suitable precursor for further synthesis. It was anticipated that the anchimeric assistance offered by the 3-*O*-acetyl group should induce stereoselective formation of the desired β-anomer. Treatment of **1** with TMSCN and $BF_3{\cdot}Et_2O$ afforded allononitrile **2** in 68% yield without detectable formation of the corresponding α-anomer. The cyano moiety was reduced with $BH_3{\cdot}Me_2S$, followed by base-mediated cleavage of the ester bonds. The primary amino group was subsequently allowed to react with $Boc_2O$ to afford *C*-glycoside **3** in a yield of 56%. Use of $NaBH_4$, $LiAlH_4$, or $NaBH_3TFA$ as reducing agents in order to achieve fully reduced products was also investigated, but those approaches proved unsuccessful due to workup difficulties.

Conversion of the primary hydroxy groups into good leaving groups by regioselective sulfonylation was attempted in order to facilitate intramolecular attack from the O3 atom. We have previously taken advantage of regioselective

  

sulfonylation and acylation procedures in our work.[27,28] Here, however, we obtained mixtures of partially sulfonylated products and our efforts to control regioselectivity turned out to be unsuccessful. This prompted the design of a new synthetic route involving protection of the primary hydroxy groups through the formation of a five-membered cyclic acetal. The reaction was carried out on *C*-glycoside **3** by treatment with 2,2-dimethoxypropane in the presence of TsOH. The crude acetal was O3-benzoylated by treatment with benzoyl chloride, affording compound **4** in 84% yield. The primary hydroxy groups were liberated by acidic hydrolysis and subsequently mesylated with mesyl chloride to give dimesylate **5** in 73% yield. Treatment of glycoside **5** with aqueous base ensured ester hydrolysis, which was followed by in situ ring closure to give the locked bicyclic C-glycoside **6** in 84% yield. Although a tedious protection-deprotection procedure had been necessary, glycoside **6** was obtained in a satisfactory overall yield of 51% from diol **3**. The remaining mesyl group was removed by treatment of **6** with NaOBz in DMF, followed by base-mediated hydrolysis of the intermediate benzoyl ester to afford glycoside **7** in 73% yield.

The need for a protecting group applicable in a *post-ON* synthetic approach resulted in the replacement of the acid-labile Boc protecting group by the base-labile Fmoc protecting group. Conversion of glycoside **7** into a suitable phosphoramidite building block furthermore implied O4-debenzylation. Acidic hydrogenolysis of glycoside **7** resulted in a fully deprotected C-glycoside and the amino functionality was subsequently Fmoc-protected under chemoselective conditions[29] to afford diol **8** in 77% yield. The primary hydroxy group was regioselectively DMT-protected by treatment with DMTCl and DIPEA in a 1:1 mixture of $CH_3CN$ and DCM. The use of this solvent mixture ensured a fast reaction without cleavage of the Fmoc group, and the DMT-protected glycoside **9** was obtained in 71% yield. Treatment of glycoside **9** with 2-cyanoethyl *N*,*N*-diisopropylphosporamidochloridite provided phosphoramidite **10** in 57% yield.

A different strategy was chosen in order to obtain a suitable SAA building block. The Boc group was replaced by a Fmoc group in order to satisfy solid-phase peptide coupling protocols, though for reasons of solubility it was considered an advantage to maintain the 4-*O*-benzyl protecting group. The Boc protecting group was removed by treatment of glycoside **7** with TFA and the crude amine was subsequently Fmoc-protected under chemoselective conditions to provide C-glycoside **11** in 86% yield. Oxidation of the primary hydroxy functionality with TEMPO and BAIB as co-oxidant was attempted.[30,31] To our surprise, the direct oxidation to the carboxylic acid turned out to be difficult to perform and we were not able to obtain a pure product. The oxidation was then carried out by conversion of the hydroxy group into an aldehyde with Dess–Martin periodinane and the aldehyde was subsequently oxidized by use of $AgNO_3$.[32] In this way, a 15% yield of the desired SAA **12** was obtained. Oxidation of the aldehyde with $NaIO_4$ and $RuCl_3$ resulted in a disappointing 14% yield. A final attempt to oxidize the aldehyde with aqueous $NaClO_2$, however, gratifyingly provided LSAA **12** in 76% yield (from **11**) after workup.

The obtained phosphoramidite **10** was used for incorporation of monomer **X** into oligonucleotides (see Exp. Sec. for details) The oligonucleotide synthesis was carried out without cleavage of the O5′-DMT group from the last monomer and the oligonucleotides were subjected to *on resin* conjugation reactions by a previously published method.[4,33] The primary amine was thus selectively deprotected with 20% piperidine in DMF and subsequently treated with pyrene-1-carboxylic acid in the presence of HOBt, HBTU, and DIPEA, producing ONs with monomer **Z** incorporated. The ONs were released from the solid support, deprotected, and purified by standard procedures to yield **ON2**, **ON9**, and **ON14**–**ON17** (see the Experimental Section for details).

## Hybridization Studies

Modified monomers that bind equally well to each of the natural bases constitute a research area of great interest. Universal bases have been used as primers for PCR reactions and as universal hybridization probes in diagnostic systems.[34] Previously published universal bases, based on a 2-deoxy-β-D-ribofuranosyl moiety, include monomer **K** (Figure 1)[6] and 3-nitropyrrole,[35–37] 5-nitroindole,[38] isocarbostyril,[39] and 8-aza-7-deazaadenine analogues.[40] These monomers showed the desired properties of displaying only small variations in the thermal stabilities ($T_m$ values) of the duplexes formed with complements containing any of the four natural bases opposite the universal base monomer, but also induced decreases in thermal stability relative to the matched unmodified DNA duplexes ($\Delta T_m$ values of –4 to –10 °C). Replacement of the nucleobase with a pyrene moiety directly attached to an LNA skeleton (**W**, Figure 1)[5] had previously been shown to produce good universal base properties, but a significant decrease in overall thermal stability relative to the unmodified matched DNA:DNA duplex containing an A:T basepair as reference (Table 1) was also observed in this case.[5]

Table 1. Thermal denaturation temperatures measured as the maxima of the first derivatives of the melting curves ($A_{260}$ vs. temperature; 5 °C to 80 °C with an increase of 1 °C min$^{-1}$) recorded in medium salt buffer ["110 mM $Na^+$"]. For buffer conditions, see General Procedure. For structures of modified monomers, see Figure 1.

| Thermal denaturation temperature ($T_m$ values) in °C | 5′-d(GCAT**H**TCAC) | | | |
|---|---|---|---|---|
| | **H** = A | **H** = T | **H** = G | **H** = C |
| **ON1** 5′-d(GTGA**T**ATGC) | 28 | 19 | 12 | 11 |
| **ON2** 5′-d(GTGA**Z**ATGC) | 21 | 21 | 19 | 20 |
| **ON3** 5′-d(GTGA**W**ATGC) | 18[8] | 19[8] | 18[8] | 17[8] |

The hybridization properties of the modified **ON2** (with monomer **Z** incorporated into a mixed 9-mer sequence) displayed a reduction in duplex stability (–7 °C) in relation to

  

the reference duplex containing an A:T basepair. However, **ON2** showed only minor variations in $T_m$ value when hybridized with any of the four natural nucleobases opposite monomer **Z**. This indicates that monomer **Z** is a good candidate as a universal base, and increases in thermal stabilities – relative to monomer **W** – were observed (Table 1).

Whether monomer **Z** was able to recognize abasic sites **Φ** when positioned opposite to these in a duplex was next examined. Pyrene recognition of abasic sites had previously been demonstrated by monomer **K**, mediated by the formation of non-hydrogen-bonded base pairs.[6,41] ONs containing an abasic site **Φ** were recognized by ONs containing monomer **Z** positioned in the complementary position to the abasic site, with a large increase in duplex stability relative to native DNA (Table 2) This can be explained by the pyrene moiety's ability to intercalate with the nucleobases, followed by enhanced base stacking. In order to achieve increased base stacking the pyrene moieties have to fit within the core of the duplex, which has been shown to be the case for monomer **K**.[6,8] However, monomer **Z** has a more rigid furanose structure with the pyrene moiety attached through an amide bond, resulting in a longer distance between the pyrene moiety and the furanose ring and possibly in a more favorable orientation of the pyrene moiety than for monomer **K**. Accordingly, putative molecular modeling showed the pyrene moiety to fit "like a hand in an glove" opposite an abasic site in a DNA:DNA duplex (Figure 2).[42]

The mixed 9-mer ONs modified with a single monomer were hybridized against each other and against complementary DNA and RNA, with an A:T(U) basepair as reference (Table 2). It was clearly seen that ONs containing an abasic site formed stable duplexes only when hybridized against ONs modified with monomer **Z** as the opposite "base", giving $T_m$ values of 22 °C (**ON5**:**ON9**) and 25 °C (**ON2**:**ON10**). No hybridization was observed when ONs with abasic sites were mixed with complementary RNA or DNA strands. These results support intercalation of the pyrene unit and provision of stability to the duplex through additional base stacking. When ONs containing incorporated monomer **Z** were hybridized against DNA, stable duplexes were obtained, although with decreases in stability relative to DNA:DNA ($\Delta T_m$ values = –5 °C and –8 °C). No duplex formation above 10 °C was observed when ONs (**ON2** and **ON9**) containing monomer **Z** were mixed with

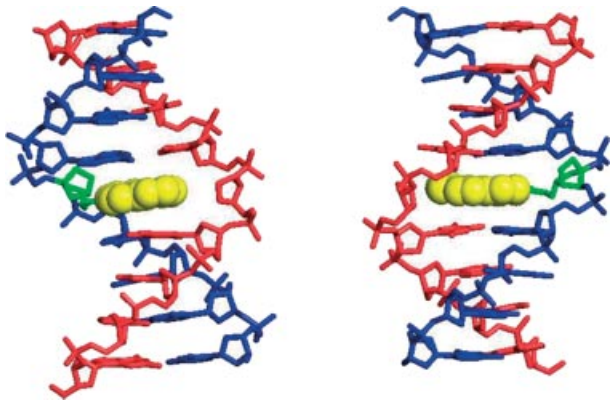

Figure 2. Model structure of **ON2**:**ON10** based on geometry optimization (Spartan02). Only modified monomers and surrounding phosphates were subjected to optimization. The unmodified nucleotides were frozen during optimization. The pyrene moiety is shown in yellow with reduced van der Waals radii and the LNA skeleton is shown in green.

complementary RNA demonstrating DNA selective binding for these ONs.

Fluorescence spectroscopy could potentially provide indirect evidence of intercalation by the pyrene moiety.[43,44] The fluorescence emission bands (Figure 3) of the duplexes obtained with **ON9** all displayed higher intensities than those of **ON9** in the single-stranded form. The fluorescence of the unhybridized **ON9** gave a fairly weak, unstructured monomer emission band, which could be the result of interactions with the neighboring nucleobases giving some quenching of the fluorescence.[43,44] When **ON9** was hybridized against the ON with an abasic site (**ON9**:**ON5**) the intensity of the fluorescence increased approximately twofold and the emission became more structured, with clear $I_1$ and $I_3$ bands indicating smaller interactions with the surrounding nucleobases. Hybridization of **ON9** against DNA (**ON1**) resulted in a strong, unstructured monomer emission band, probably due to a dynamic equilibrium between different orientations of the pyrene moiety, one being the intercalated form, probably with the opposing adenine base flipped out of the duplex, and another being one in which the pyrene moiety is flipped out of the duplex, explaining the increased fluorescence. With two pyrene moieties opposite each other (**ON9**:**ON2**), the intensity is almost the same as or slightly lower than the intensity seen with one pyrene

Table 2. Thermal denaturation temperatures measured as the maxima of the first derivatives of the melting curves ($A_{260}$ vs. temperature; 5 °C to 80 °C with an increase of 1 °C min$^{-1}$) recorded in medium salt buffer ["110 mM $Na^+$"]. For structures of modified monomers, see Figure 1. For buffer conditions, see General Procedure.

| | Thermal denaturation temperatures ($T_m$ values) measured in °C | | | | |
|---|---|---|---|---|---|
| | **ON7** d(GCATATCAC) | **ON8** r(GCAUAUCAC) | **ON9** d(GCAT**Z**TCAC) | **ON10** d(GCAT**Φ**TCAC) | **ON11** d(GCAT**X**TCAC) |
| **ON1** d(GTGATATGC) | 29 | 27 | 21 | <10 | <10 |
| **ON4** r(GUGAUAUGC) | 28 | 37 | <10 | <10 | <10 |
| **ON2** d(GTGA**Z**ATGC) | 24 | <10 | 21 | 25 | 25 |
| **ON5** d(GTGA**Φ**ATGC) | <10 | <10 | 22 | <10 | <10 |
| **ON6** d(GTGA**X**ATGC) | <10 | <10 | 20 | <10 | <10 |

  

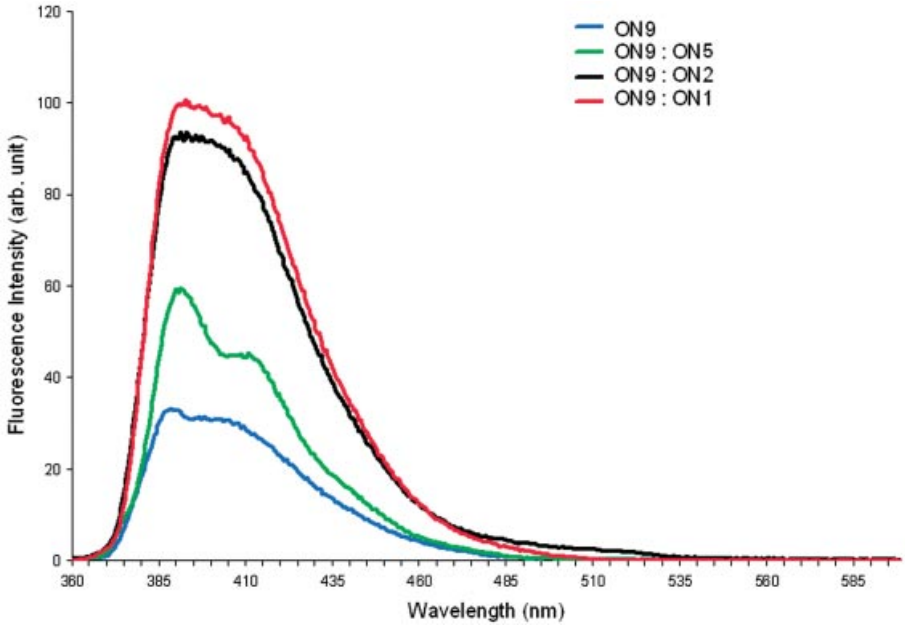


Figure 3. Steady-state fluorescence spectra of **ON9** with 0.15 μM of each strand recorded in air-saturated medium salt buffer ["110 mM $Na^+$"] at 19 °C.

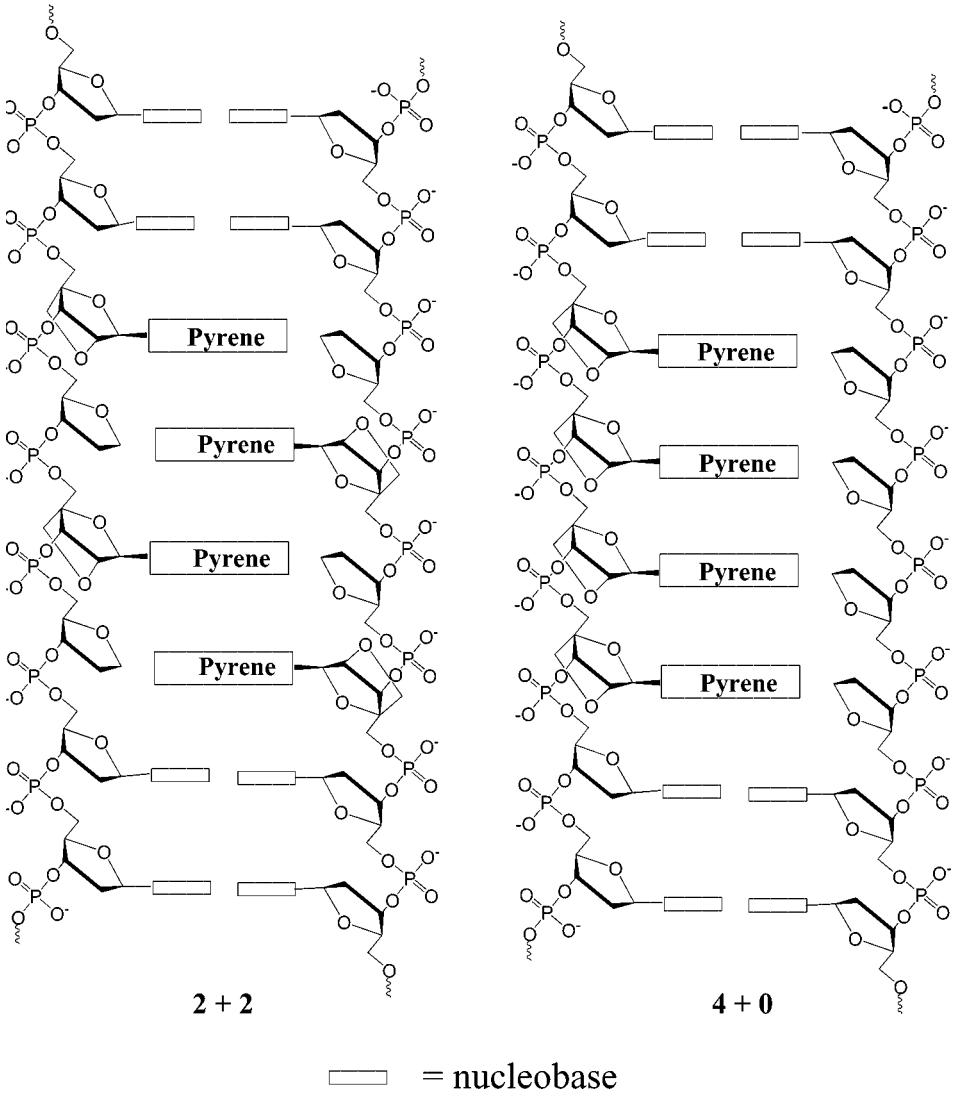


Figure 4. The "2+2" and "4+0" systems.

  

moiety hybridized against DNA (**ON9**:**ON1**), and displaying an unstructured band. This observation can only be explained in terms of dynamic equilibrium of the two pyrene moieties interchanging as intercalating units. The two pyrene moieties would not be anticipated both to intercalate simultaneously since this would probably result in the formation of an excimer band in the fluorescence emission spectrum, which is not observed.

The relative stable duplexes formed by ONs modified with a single monomer **Z** (**ON2** and **ON9**) positioned opposite to abasic sites **Φ** prompted us to expand into more complex systems (Figure 4 and Table 3). We therefore designed two systems containing four abasic sites with monomer **Z** placed opposite these. In the first system, four **Z** monomers were placed consecutively in the same strand (the "4+0" system). Alternatively, two **Z** monomers alternating with two abasic sites **Φ** were positioned in each of the complementary strands (the "2+2" system). As described earlier, *post-ON* synthesis conveniently provided us with **ON14**–**ON17**. The stabilities of the corresponding duplexes were investigated by thermal denaturation studies (Table 3), and the orientations of the pyrene moieties were investigated by fluorescence spectroscopy.

**ON12**: 5'-d(GTG-ATT-TAG-CGA) **ON13**: 5'-d(TCG-CTA-AAT-CAC)

**ON14**: 5'-d(GTG-AT**Z**-**ΦZΦ**-AGC-GA) **ON15**: 5'-d(TCG-CT**Z**-**ΦZΦ**-ATC-AC)

**ON16**: 5'-d(GTG-AT**Z**-**ZZZ**-AGC-GA) **ON17**: 5'-d(TCG-CT**Φ**-**ΦΦΦ**-ATC-AC)

The presence of pyrenes and abasic monomers placed in a "2+2 zipper"-like manner (**ON14**:**ON15**) gave a decrease in the $T_m$ value of 15 °C relative to the duplex with native A:T basepairs. A larger decrease of 21 °C was observed when all four pyrene moieties were placed in one strand opposite four consecutive abasic sites (**ON16**:**ON17**). However, the notable point is the fact that the ONs modified with monomer **Z** are able to form stable duplexes targeting abasic sites, which is not the case for the corresponding DNA strand containing a DNA thymine monomer instead of the **Z** monomer. The best way to obtain stable duplexes is also shown to be the zipper model, rather than by placing all the pyrene moieties in one strand. A very similar system using monomer **K** has been published independently of us, but resulted in the formation of a substantially less stable complex.[45]

Table 3. For conditions, see Table 1.

| | Thermal denaturation temperatures ($T_m$ values) measured in °C | | |
|---|---|---|---|
| | **ON12** | **ON14** | **ON16** |
| **ON13** | 43 | | |
| **ON15** | | 28 | |
| **ON17** | | | 22 |

We investigated whether the pyrene moieties were positioned in a sandwich-like fashion by fluorescence emission spectroscopy, as induction of excimer band emission would be expected (see Figure 5).[46] In the system with four consecutively placed pyrenes ("4+0") the fluorescence spectra in the hybridized form and in the unhybridized form are virtually identical displaying similar excimer band intensities, with the only difference being a stronger monomer fluorescence in the hybridized form. This shows that the pyrenes are located in close proximity to each other in both the hybridized and unhybridized forms, but does not provide any information relating to the orientations of the pyrene moieties within the duplex. In the spectra of the systems containing four pyrene moieties placed in a "2+2 zipper", the intensity of the monomer fluorescence of the duplex is identical to the monomer fluorescence intensity of

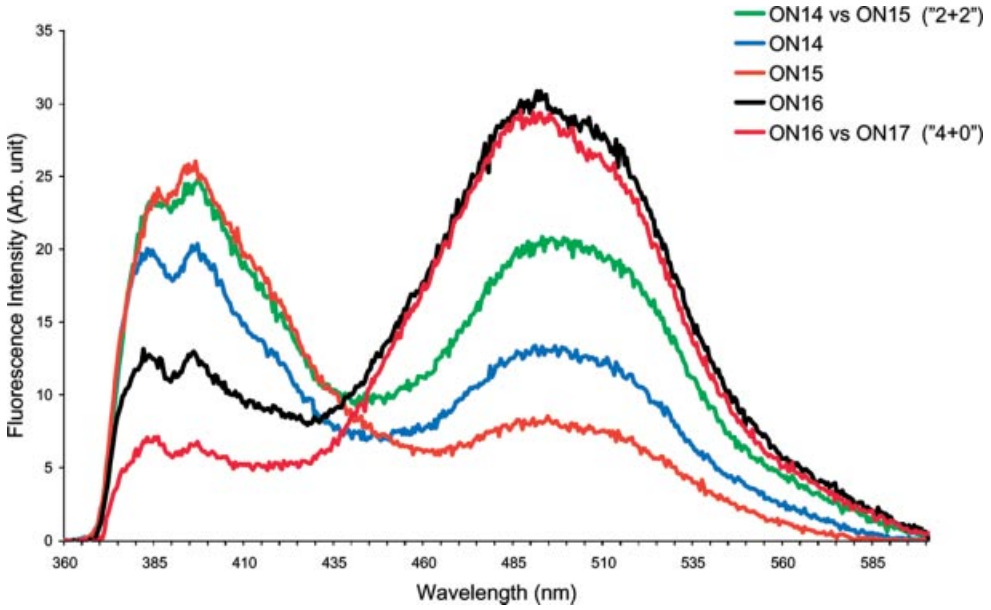


Figure 5. Steady-state fluorescence spectra with 0.15 μM of each strand recorded in air-saturated medium salt buffer ["110 mM $Na^+$"] at 19 °C.

  

unhybridized **ON15** and only marginally stronger than the monomer fluorescence intensity of unhybridized **ON14**. The excimer band originating from the duplex is of the same intensity as the combined excimer bands of the two strands in their single-stranded forms. No enhanced intensity of the excimer band of the fluorescence of the duplex relative to the excimer fluorescence band of the combined ssONs' (**ON14** + **ON15**) fluorescence is observed. The firm conclusion that can be drawn from these experiments is that the pyrene units remain within a few Ångstrøms distance in both the "2+2" and the "4+0" duplex systems, as testified by the significant excimer bands. This indicates that the pyrene moieties are positioned in a sandwich-like manner within the core of the duplexes.

## Conclusion

The synthesis of the conformationally locked aminomethyl *C*-glycoside monomer **X** and its incorporation into oligonucleotides have been achieved. The potential of this monomer as a very useful conjugation site for the introduction of functional groups within the core of a DNA:DNA duplex was demonstrated by the unprecedented introduction of four identical groups by a *post-ON* synthetic approach. The pyrene-functionalized aminomethyl *C*-glycoside monomer **Z** demonstrated versatility by functioning as a universal base with a satisfactory affinity towards DNA. ONs incorporating monomer **Z** show no hybridization with RNA, but are able to recognize DNA strands containing abasic sites **Φ** positioned opposite to monomer **Z**. This ability was utilized for the creation of fairly complex systems that, by virtue of the bicyclic core structure in combination with the short aminomethyl linkage, are stabilized relative to similar systems using a monocyclic DNA scaffold. This is evidence for the utility of bicyclic LNA-like monomers for the construction of complicated and unnatural nucleic acid systems, which may find applicability in nanotechnology.

## Experimental Section

All reagents were obtained from commercial suppliers and were used without further purification. Reactions were carried out under nitrogen or argon when anhydrous solvents were used. Column chromatography was performed with Silica gel 60 (particle size 0.040–0.063 μm, Merck). Dichloromethane (DCM) used for column chromatography was distilled prior to use. TLC analysis was conducted with Fluka silica gel 60 $F_{254}$ alumina sheets. UV absorbing compounds were detected at 254 nm and charred either with 20% $H_2SO_4$ in ethanol or with $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g), $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g), and $H_2SO_4$ (100 mL) in $H_2O$ (900 mL). Unsaturated hydrocarbons and aldehydes were sprayed with $KMnO_4$ (2%)/$K_2CO_3$ (1%) in $H_2O$. Amines were charred with ninhydrin (0.3 g) and acetic acid (3 mL) in 1-butanol (100 mL). NMR spectra were recorded with a Varian Unity 300 spectrometer. Chemical shift values are given relative to tetramethylsilane as internal standard ($^1$H and $^{13}$C NMR) and relative to 85% $H_3PO_4$ as external reference ($^{31}$P NMR). Coupling constants (*J* values) are given in Hz. Assignments of NMR spectra are based on 2D spectra and follow the standard carbohydrate and von Baeyer nomenclature. Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) was performed on a 4.7 Tesla Ultima (IonSpec, Irvine, CA) Fourier transform ion cyclotron resonance (FTICR) mass spectrometer.

**3-*O*-Acetyl-2,5-anhydro-6-*O*-benzoyl-5-*C*-[(benzoyloxy)methyl]-4-*O*-benzyl-D-allononitrile (2):** TMSCN (475 μL, 3.56 mmol) and $BF_3{\cdot}Et_2O$ (250 μL, 1.96 mmol) were added to a stirred solution of **1**[26] (990 mg, 1.75 mmol) in anhydrous acetonitrile (9.0 mL). The reaction mixture was stirred for 30 min at room temp. before addition of a sat. aq. solution of $NaHCO_3$ (10 mL). The mixture was stirred for 15 min at room temp. and extracted with ethyl acetate (50 mL). The organic phase was washed successively with sat. aq. solutions of $NaHCO_3$ (3×25 mL, 10%) and brine (25 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate in petroleum ether [0% → 25% (v/v)] as eluent, yielding the allononitrile **2** (635 mg, 68%). $^1$H NMR ($CDCl_3$): $\delta$ = 8.04–8.00 (m, 4 H, $H_{arom}$), 7.58–7.55 (m, 2 H, $H_{arom}$), 7.46–7.40 (m, 4 H, $H_{arom}$), 7.26–7.20 (m, 5 H, $H_{arom}$), 5.68 (dd, *J* = 5.2 Hz, *J* = 2.7 Hz, 1 H, 3-H), 4.88 (d, *J* = 12.4 Hz, 1 H, C$H_a$H$_b$), 4.87 (d, *J* = 2.4 Hz, 1 H, 4-H), 4.66 (d, *J* = 12.2 Hz, 1 H, C$H_a$H$_b$′), 4.64 (d, *J* = 11.3 Hz, 1 H, C$H_a$H$_b$″), 4.59 (d, *J* = 5.2 Hz, 1 H, 2-H), 4.50 (m, 1 H, CH$_a$$H_b$″), 4.48 (d, *J* = 11.4 Hz, 1 H, CH$_a$$H_b$′), 4.44 (d, *J* = 12.6 Hz, 1 H, CH$_a$$H_b$), 2.18 (s, 3 H, $CH_3$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 169.4, 166.1, 166.0 (CO), 136.2, 133.4, 133.3, 129.8, 129.7, 128.6, 128.5, 128.1 (Ar), 116.0 (CN), 84.7 (C-2), 78.3, 74.2, 74.0, 69.0, 64.0, 63.4 (C-3, C-4, C-5, C-6, C-6′, *C*H$_2$-Bn), 20.7 ($CH_3$) ppm. HRMS: calcd. for $C_{30}H_{27}NO_8Na$ 552.1629; found *m*/*z* 552.1630.

**2,5-Anhydro-4-*O*-benzyl-1-[(*tert*-butoxycarbonyl)amino]-1-deoxy-5-*C*-(hydroxymethyl)-D-allitol (3):** $BH_3{\cdot}S(Me)_2$ in THF (2 M, 3.4 mL, 6.8 mmol) was slowly added to a stirred and cooled (0 °C) solution of **2** (362 mg, 0.684 mmol) in freshly distilled THF (3.5 mL). The reaction mixture was stirred for 16 h at room temp. The reaction mixture was cooled (0 °C), followed by dropwise addition of aq. HCl (6 M, 7 mL, 42 mmol). The mixture was stirred for 30 min at room temp. and the solvents were evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of EtOH and 2 M aq. NaOH (1:1, v/v, 10 mL, 10 mmol) and was stirred for 2 h at room temp. DCM (10 mL) and $Boc_2O$ (200 μL, 0.871 mmol) were added to the reaction mixture and stirring was continued for another 16 h at room temp. The mixture was extracted with ethyl acetate (3×15 mL) and the combined organic phase was washed successively with a sat. aq. solution of $NaHCO_3$ (3×15 mL) and brine (15 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH in EtOAc [0% → 10% (v/v)] as eluent, yielding the allitol **3** (145 g, 56%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.35–7.31 (m, 5 H, $H_{arom}$), 4.73 (d, *J* = 11.2 Hz, 1 H, C$H_a$H$_b$), 4.56 (d, *J* = 11.3 Hz, 1 H, CH$_a$$H_b$), 4.38 (m, 1 H, 4-H), 4.03 (d, *J* = 8.6 Hz, 1 H, 2-H), 3.97 (m, 2 H, 3-H, C$H_a$H$_b$′), 3.77 (d, *J* 11.4 Hz, 1 H, C$H_a$H$_b$″), 3.57–3.29 (m, 3 H, CH$_a$$H_b$′, CH$_a$$H_b$″, C$H_a$H$_b$‴), 3.09–3.04 (m, 1 H, CH$_a$$H_b$‴), 1.44 (s, 9 H, C(CH$_3$)$_3$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 157.2 (CO), 137.5, 128.6, 128.1, 127.8 (Ar), 87.0 (C-5), 85.3 (C-4), 80.0 ($C_q$, Boc), 78.1 (C-2), 73.1 (C-3), 71.9 (*C*H$_2$Bn), 63.3, 62.5 (C-6, C-6′), 43.5 (C-1), 28.3 (C(*C*H$_3$)$_3$) ppm. HRMS: calcd. for $C_{19}H_{29}NO_7Na$ 406.1836; found *m*/*z* 406.1821.

**2,5-Anhydro-3-*O*-benzoyl-4-*O*-benzyl-1-[(*tert*-butoxycarbonyl)amino]-1-deoxy-5-*C*-hydroxymethyl-6,5-(*C*-hydroxymethyl)-di-*O*-iso-**

  

**propylidene-D-allitol (4):** A catalytic amount of *p*-toluenesulfonic acid (50 mg, 0.29 mmol) and 2,2-dimethoxypropane (29.0 mL, 181 mmol) were added to a stirred solution of **3** (8.86 g, 23.1 mmol) in anhydrous THF (400 mL). The reaction mixture was stirred for 2 h at room temp. before addition of a sat. aq. solution of $NaHCO_3$ (25 mL) and DCM (300 mL). The resulting mixture was stirred for 5 min at room temp., after which the phases were separated and the organic phase was washed successively with a sat. aq. solution of $NaHCO_3$ (3×150 mL) and brine (150 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in anhydrous DCM (400 mL) and the solution was cooled to 0 °C, followed by addition of benzoyl chloride (8.7 mL, 75 mmol) and TEA (14 mL, 100 mmol). The reaction mixture was stirred for 15 min at 0 °C. The mixture was allowed to reach room temperature and then stirred for another 16 h at room temp. before addition of a sat. aq. solution of $NaHCO_3$ (25 mL). The organic phase was separated and washed successively with a sat aq. solution of $NaHCO_3$ (3×150 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate and petroleum ether [0% → 30% (v/v)] as eluent, yielding the allitol **4** (10.25 g, 84%). $^1$H NMR ($CDCl_3$): $\delta$ = 8.12–8.09 (m, 1 H, $H_{arom}$), 8.03–8.00 (m, 1 H, $H_{arom}$), 7.61–7.41 (m, 4 H, $H_{arom}$), 7.22 (m, 4 H, $H_{arom}$), 5.29–5.26 (m, 1 H, 3-H), 4.62 (d, $J$ = 11.5 Hz, 1 H, C$H_a$H$_b$), 4.57 (d, $J$ = 11.4 Hz, 1 H, CH$_a$$H_b$), 4.29 (dd, $J$ = 4.9 Hz and $J$ = 10.3 Hz, 1 H, 2-H), 4.24 (d, $J$ = 12.3 Hz, 1 H, C$H_a$H$_b$′), 4.03 (d, $J$ = 5.0 Hz, 1 H, 4-H), 3.91 (d, $J$ = 12.2 Hz, 1 H, CH$_a$$H_b$′), 3.76–3.68 (m, 2 H, C$H_a$H$_b$″ and CH$_a$$H_b$″), 3.45–3.20 (m, 2 H, C$H_a$H$_b$‴ and CH$_a$$H_b$‴), 1.43 (m, 15 H, C(CH$_3$)$_3$, 2×CH$_3$, isoprop) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 165.7 (CO), 137.4, 133.4, 129.8, 129.4, 128.5, 128.4, 128.3, 128.1 (Ar), 98.2 ($C$(CH$_3$)$_2$), 79.5, 79.4, 78.4, 74.1, 73.1 (C-5, C-4, C-3, C-2, $C$(CH$_3$)$_3$, $C$H$_2$Bn), 66.4, 62.7 (C-6, C-6′), 42.5 (C-1), 28.3 (C($C$H$_3$)$_3$), 23.9, 23.2 (2×C($C$H$_3$)$_2$) ppm. HRMS: calcd. for $C_{29}H_{37}NO_8Na$ 550.2411; found *m*/*z* 550.2420.

**2,5-Anhydro-3-*O*-benzoyl-4-*O*-benzyl-1-[(*tert*-butoxycarbonyl)-amino]-1-deoxy-6-*O*-(methylsulfonyl)-5-*C*-[(methylsulfonyloxy)-methyl]-D-allitol (5):** *p*-Toluenesulfonic acid (760 mg, 4.80 mmol) was added to a stirred solution of **4** (10.25 g, 19.43 mmol) in MeOH (200 mL). After 45 min, a sat. aq. solution of $NaHCO_3$ (25 mL) was added and stirring was continued for 15 min. The mixture was concentrated to dryness by evaporation under reduced pressure and ethyl acetate (300 mL) was added to the resulting residue. The separated organic phase was washed successively with a sat. aq. solution of $NaHCO_3$ (3×100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in anhydrous DCM (250 mL) and cooled to 0 °C. MsCl (7.7 mL, 100 mmol) and TEA (16.7 mL, 120 mmol) were added to the resulting mixture. After 3.5 h of stirring, a sat. aq. solution of $NaHCO_3$ (25 mL) was added to the reaction mixture. The mixture was stirred for 15 min and was then concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (300 mL) and the resulting mixture washed successively with a sat. aq. solution of $NaHCO_3$ (3×100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate in petroleum ether [0% → 50% (v/v)] as eluent, yielding allitol **5** (9.20 g, 73%). $^1$H NMR ($CDCl_3$): $\delta$ = 8.07–8.04 (m, 2 H, $H_{arom}$), 7.61 (t, $J$ = 7.4 Hz, 1 H, $H_{arom}$), 7.47 (t, $J$ = 7.7 Hz, 2 H, $H_{arom}$), 7.29–7.20 (m, 5 H, $H_{arom}$), 5.48 (t, $J$ = 4.2 Hz, 1 H, 3-H), 5.06 (m, 1 H, NH), 4.77 (d, $J$ = 11.7 Hz, 1 H, C$H_a$H$_b$), 4.64 (d, $J$ = 11.1 Hz, 1 H, C$H_a$H$_b$′), 4.46 (d, $J$ = 11.2 Hz, 1 H, CH$_a$$H_b$′), 4.40–4.35 (m, 2 H, 4-H and C$H_a$H$_b$″), 4.32 (d, $J$ = 5.6 Hz, 1 H, 2-H), 4.27 (d, $J$ = 11.6 Hz, 1 H, CH$_a$$H_b$), 4.18 (d, $J$ = 11.0 Hz, 1 H, CH$_a$$H_b$″), 3.42 (m, 2 H, C$H_a$H$_b$‴ and CH$_a$$H_b$‴), 3.03 (s, 3 H, Ms), 3.01 (s, 3 H, Ms) 1.45 (s, 9 H, C(CH$_3$)$_3$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 165.5, 156.0 (CO), 136.6, 133.6, 129.8, 129.1, 128.6, 128.5, 128.3 (Ar), 82.3, 81.5, 78.4, 74.2, 72.7 (C-5, C-4, C-3, C-2, $C$(CH$_3$)$_3$, $C$H$_2$Bn) 68.1, 67.6 (C-6, C-6′), 42.3 (C-1), 37.9 (Ms), 28.3 (C($C$H$_3$)$_3$) ppm. MALDI-MS: *m*/*z* 666.2 [M + Na]$^+$. HRMS: calcd. for $C_{28}H_{37}NO_{12}S_2Na$ 666.1649; found *m*/*z* 666.1648.

**(1*R*,3*S*,4*S*,7*S*)-7-Benzyloxy-3-[(*tert*-butoxycarbonyl)aminomethyl]-1-[(methylsulfonyloxy)methyl]-2,5-dioxabicyclo[2.2.1]-heptane (6):** Aq. NaOH (4 M, 150 mL, 0.6 mol) was added to a stirred solution of **5** (9.27 g, 14.8 mmol) in THF (150 mL). The reaction mixture was stirred for 16 h at room temp., after which the mixture was extracted with ethyl acetate (3×150 mL). The combined organic phase was collected, washed with brine (50 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with ethyl acetate in petroleum ether [0% → 50% (v/v)] as eluent, yielding **6** (5.36 g, 84%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.38–7.35 (m, 5 H, $H_{arom}$), 4.80 (m, 1 H, NH), 4.70 (d, $J$ = 11.6 Hz, 1 H, C$H_a$H$_b$), 4.57 (d, $J$ = 11.6 Hz, 1 H, CH$_a$$H_b$), 4.49 (d, $J$ = 10.3 Hz, 1 H, C$H_a$H$_b$′), 4.40 (d, $J$ = 11.7 Hz, 1 H, CH$_a$$H_b$′), 4.29 (s, 1 H, 7-H), 4.09–4.02 (m, 3 H, C$H_a$H$_b$″, 3-H, 4-H), 3.90 (d, $J$ = 7.8 Hz, 1 H, CH$_a$$H_b$″), 3.14–3.03 (m, 5 H, C$H_a$H$_b$‴, CH$_a$$H_b$‴ and CH$_3$), 1.44 (s, 9 H, C(CH$_3$)$_3$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 156.1 (CO) 137.3, 128.5, 128.4, 127.8 (Ar), 84.0 (C-1), 83.1 (C-3), 78.5 (CH$_2$), 77.5 (C-7), 72.5, 72.1 (CH$_2$, C-4) 66.2 (CH$_2$), 42.3 (CH$_2$), 37.7 (CH$_3$), 28.3 (C($C$H$_3$)$_3$, Boc) ppm. MALDI-MS: *m*/*z* 466.1 [M + Na]$^+$. HRMS: calcd. for $C_{19}H_{29}NO_7Na$ 466.1506; found *m*/*z* 466.1486.

**(1*S*,3*S*,4*S*,7*S*)-7-Benzyloxy-3-[(*tert*-butoxycarbonyl)aminomethyl]-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (7):** NaOBz (2.67 g, 18.5 mmol) was added to a stirred solution of **6** (5.20 g, 11.7 mmol) in anhydrous DMF (200 mL). The reaction mixture was stirred for 14 h at 100 °C, was allowed to reach room temp., and was subsequently filtered. EtOAc (250 mL) was added to the filtrate and the resulting mixture was washed successively with $H_2O$ (3×200 mL) and brine (2×150 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in a mixture of ethanol (125 mL) and aq. NaOH (4 M, 125 mL, 0.5 mol) and stirred for 2 h at room temp. Ethyl acetate (200 mL) was added and the organic phase was separated, washed successively with $H_2O$ (2×100 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by flash silica gel column chromatography with ethyl acetate in petroleum ether [0% → 20% (v/v)] as eluent, yielding **7** (3.12 g, 73%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.36 (m, 5 H, $H_{arom}$, Bn), 4.91 (br. s, 1 H, NH), 4.67 (d, $J$ = 10.2 Hz, 1 H, C$H_a$H$_b$), 4.62 (d, $J$ = 9.9 Hz, 1 H, CH$_a$$H_b$), 4.20 (s, 1 H, 7-H), 4.08–4.01 (m, 2 H, C$H_a$H$_b$′, 3-H), 3.85–3.79 (m, 2 H, CH$_a$$H_b$′, 4-H), 3.37 (m, 2 H, C$H_a$H$_b$″ and CH$_a$$H_b$″), 3.19–3.13 (m, 1 H, C$H_a$H$_b$‴), 3.06–2.97 (m, 1 H, CH$_a$$H_b$‴), 1.47 (s, 9 H, C(CH$_3$)$_3$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 156.3 (CO), 137.5, 128.4, 127.8, 127.6 (Ar), 86.6 (C-1), 82.6 (C-3), 79.8 ($C$(CH$_3$)$_3$), 78.2 (CH$_2$), 77.6 (C-7), 72.6 (C-6, rotamer), 72.0 (C-4), 69.2 (C-6, rotamer), 58.6 ($C$H$_2$), 42.5 (CH$_2$), 28.3 (C($C$H$_3$)$_3$) ppm. HRMS: calcd. for $C_{19}H_{27}NO_6Na$ 388.1731; found *m*/*z* 388.1726.

**(1*S*,3*S*,4*S*,7*S*)-3-{[(Fluorenylmethoxy)carbonyl]aminomethyl}-7-hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (8):** HCl [37% (wt.-%), five drops] and a catalytic amount of Pd on charcoal (10 wt.-%, 20 mg) were added to a solution of **7** (1.50 g, 4.11 mmol)

  

in ethanol (40 mL). $N_2$ was bubbled through the solution for 5 min, after which $H_2$ was flushed through the system. The reaction mixture was stirred for 14 h at room temp. under an atmosphere of $H_2$. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of THF and $H_2O$ (4:1 v/v, 50 mL) and MgO (0.82 g, 20.5 mmol) was added. The resulting mixture was cooled to 0 °C and stirred for 5 min before addition of FmocCl (3.12 g, 12.3 mmol). The reaction mixture was stirred for 1 h at 0 °C followed by filtration. The residue was washed with EtOAc (50 mL) and the combined filtrate was evaporated to dryness under reduced pressure. The residue was purified by flash silica gel column chromatography with MeOH in DCM [0% → 10% (v/v)] as eluent, yielding **8** (1.26 g, 77%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.73 (d, $J$ = 7.6 Hz, 2 H, $H_{arom}$), 7.54 (d, $J$ = 7.4 Hz, 2 H, $H_{arom}$), 7.38 (t, $J$ = 7.6 Hz, 2 H, $H_{arom}$), 7.27 (t, $J$ = 7.4 Hz, 2 H, $H_{arom}$), 5.54–5.50 (m, 1 H, NH), 4.36 (d, $J$ = 6.4 Hz, 2 H, $CH_{2,Fmoc}$) 4.21 (s, 1 H, 4-H), 4.14 (t, $J$ = 6.4 Hz, 1 H, $CH_{Fmoc}$) 4.09 (s, 1 H, 7-H), 3.98 (t, $J$ = 6.3 Hz, 1 H, 3-H), 3.92 (d, $J$ = 8.2 Hz, 1 H, C$H_a$CH$_b$), 3.26–3.19 (m, 3 H, $CH_2$, $CH_aH_b$), 3.26–3.19 (m, 1 H, C$H_a$H$_b$N), 3.10–3.01 (m, 1 H, $CH_aH_b$N) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 157.0 (CO), 143.7, 141.2, 127.7, 127.0, 124.9, 120.0 (Ar), 87.1 (C-1), 81.7 (C-3), 80.1 (C-7), 71.8 (C-6), 71.6 (C-4), 66.8 ($CH_{Fmoc}$), 58.0 ($CH_2$), 47.0 ($CH_{Fmoc}$), 42.9 ($CH_2$) ppm. HRMS: calcd. For $C_{22}H_{23}NO_6Na$ 420.1418; found *m*/*z* 420.1418.

**(1*R*,3*S*,4*S*,7*S*)-1-[(4,4′-Dimethoxytrityl)oxymethyl]-3-{[(fluorenylmethoxy)carbonylamino]methyl}-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (9):** TEA (320 μL, 2.30 mmol) and DMTCl (560 mg, 1.65 mmol) were added to a solution of **8** (600 mg, 1.51 mmol) in a mixture of anhydrous DCM and anhydrous acetonitrile (1:1 v/v, 30 mL). The reaction mixture was stirred for 1.5 h at room temp., after which $H_2O$ (25 mL) and ethyl acetate (30 mL) were added. The organic phase was separated and washed successively with a sat. aq. solution of $NaHCO_3$ (3 × 25 mL) and brine (25 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with TEA and ethyl acetate in petroleum ether [1%, 1% → 40% (v/v/v)] as eluent, yielding **9** (0.740 g, 71%) . $^1$H NMR ($CDCl_3$): $\delta$ = 7.76–7.74 (m, 2 H, $H_{arom}$), 7.58–7.56 (m, 2 H, $H_{arom}$), 7.44–7.20 (m, 13 H, $H_{arom}$), 6.84–6.81 (m, 4 H, $H_{arom}$), 5.00 (br. s, 1 H, NH), 4.42–4.40 (m, 2 H, C$H_a$H$_b$ and $CH_aH_b$), 4.18–4.08 (m, 1 H, $CH^{Fmoc}$), 4.06–3.99 (m, 6 H, C$H_a$H$_b$′, $CH_aH_b$′, $CH_2^{Fmoc}$, 3-H and 7-H), 3.77 (s, 6 H, OMe), 3.42–3.33 (m, 2 H, C$H_a$H$_b$′′ and 4-H), 3.08–3.04 (m, 1 H, $CH_aH_b$′′) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 158.5 (CO), 149.8, 144.5, 143.8, 141.3, 135.6, 135.5, 130.0, 128.1, 127.9, 127.7, 127.1, 126.9, 125.0, 120.0, 113.2, (Ar), 86.3, 85.9, (C-1, $C_q$ DMT), 81.4 (C-3), 80.2 (C-7), 73.7, 72.4 ($CH_2$, C-4), 66.9 ($CH_2^{Fmoc}$), 60.8 ($CH^{Fmoc}$), 55.2 (OMe), 47.2 (C-6) 43.0 ($CH_2$) ppm. HRMS: calcd. For $C_{43}H_{41}NO_8Na$: 722.2724; found *m*/*z* 722.2740.

**(1*R*,3*S*,4*S*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphanyloxy]-1-[(4,4′-dimethoxytrityl)oxymethyl]-3-{[(fluorenylmethoxy)carbonylamino]methyl}-2,5-dioxabicyclo[2.2.1]heptane (10):** DIPEA (0.9 mL) and 2-cyanoethyl *N*,*N*-diisopropylphosporamidochloridite (200 μL, 0.965 mmol) were added to a solution of **9** (575 mg, 0.823 mmol) in anhydrous DCM (2.6 mL). The reaction mixture was stirred for 1 h at room temp. before addition of ethyl acetate (10 mL) and $H_2O$ (5 mL). The organic phase was separated and washed successively with a sat. aq. solution of $NaHCO_3$ (3 × 5 mL) and brine (5 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with TEA and ethyl acetate in petroleum ether [1%, 1% → 29% (v/v/v)] as eluent, yielding the phosphoramidite **10** (423 mg, 57%). $^{31}$P NMR: ([D$_6$]DMSO), $\delta$ = 150.3, 149.5 ppm. HRMS: calcd. for $C_{52}H_{58}N_3O_9PNa$: 922.3803; found *m*/*z* 922.3808.

**(1*S*,3*S*,4*S*,7*S*)-7-Benzyloxy-3-{[(fluorenylmethoxy)carbonylamino]methyl}-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (11):** A solution of **7** (500 mg, 1.37 mmol) in TFA (5 mL) was stirred for 30 min. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in a mixture of THF and $H_2O$ (4:1 v/v, 20 mL) and was cooled to 0 °C. MgO (270 mg, 6.70 mmol) was added and the mixture was stirred for 5 min at 0 °C, followed by addition of FmocCl (390 mg, 1.51 mmol). The resulting mixture was stirred for 1 h at 0 °C, and $H_2O$ (5 mL) and ethyl acetate (20 mL) were added. The separated organic phase was washed with sat. aq. $NaHCO_3$ (3 × 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by flash silica gel column chromatography with MeOH and DCM [0% → 10% (v/v)] as eluent, yielding **11** (577 mg, 86%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.75–7.24 (m, 13 H, $H_{arom}$), 5.14 (br. s, 1 H, NH), 4.65–4.53 (m, 2 H, C$H_a$H$_b$ and $CH_aH_b$), 4.46–4.33 (m, 2 H, $CH_2^{Fmoc}$), 4.19–4.15 (m, 2 H, 7-H, $CH^{Fmoc}$), 4.05–3.99 (m, 3 H, C$H_a$H$_b$′, $CH_aH_b$′ and 3-H), 3.84–3.78 (m, 3 H, C$H_a$H$_b$′′, $CH_aH_b$′′ and 4-H), 3.25–3.18 (m, 1 H, C$H_a$H$_b$′′′), 3.06–2.97 (m, 1 H, $CH_aH_b$′′′) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 156.9 (CO), 143.8, 141.4, 137.6, 128.5, 128.0, 127.8, 127.7, 127.1, 125.0, 125.0, 120.1 (Ar), 86.7 (C-1), 82.5 (C-3), 78.2 ($CH_2$), 77.7 (C-7), 72.7, 72.1, 66.9 ($CH_2^{Fmoc}$), 58.7 (C-6), 47.2, ($CH^{Fmoc}$), 43.1 ($CH_2$) ppm. HRMS: calcd. for $C_{29}H_{29}NO_6Na$: 510.1887; found *m*/*z* 510.1871.

**(1*S*,3*S*,4*S*,7*S*)-7-Benzyloxy-3-{[(fluorenylmethoxy)carbonylamino]methyl}-2,5-dioxabicyclo[2.2.1]heptane-1-carboxylic Acid (12):** Dess–Martin periodinane (600 mg, 1.41 mmol) was added to a solution of **11** (458 mg, 0.94 mmol) in anhydrous DCM (15 mL). The reaction mixture was stirred for 3 h at room temp., after which ethyl acetate (20 mL), a sat. aq. solution of $NaHCO_3$, and 10% (wt.-%) aq. $Na_2S_2O_3$ (1:1 v/v, 20 mL) were added. The resulting mixture was stirred at room temp. until both phases became clear. The organic phase was separated, washed successively with sat. aq. $NaHCO_3$ (3 × 10 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in a mixture of *t*BuOH (10 mL) and 2-methylbut-2-ene (1 mL) followed by addition of aq. $NaH_2PO_4$ (5.05 M, 10 mL, 50.5 mmol). To this solution was added $NaClO_2$ (914 mg, 10.1 mmol, in small portions over 15 min). The reaction mixture was stirred vigorously for 2 h and then cooled to 0 °C, aq. HCl (1 M, 20 mL, 20 mmol) was added, and extraction was performed with $CHCl_3$ (3 × 20 mL). The combined organic phase was dried ($MgSO_4$), filtered, and concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with acetic acid in ethyl acetate [1%, (v/v)] as eluent, yielding **12** (358 mg, 76%). $^1$H NMR ($CD_3OD$): $\delta$ = 7.77–7.23 (m, 13 H, $H_{arom}$), 4.56 (br. s, 2 H, C$H_a$H$_b$ and $CH_aH_b$), 4.42–3.95 (m, 8 H, $CH_{Fmoc}$, $CH_{2Fmoc}$, C$H_a$H$_b$′, $CH_aH_b$′, C$H_a$H$_b$′′, $CH_aH_b$′′, 3-H, 4-H, 7-H), 3.17–3.07 (m, 2 H, C$H_a$H$_b$′′′ and $CH_aH_b$′′′) ppm. $^{13}$C NMR ($CD_3OD$): $\delta$ = 170.6 (COOH), 158.8 (CONH), 145.2, 142.6, 138.9, 129.4, 128.8, 128.7, 128.1, 126.2, 126.1, 120.9 (Ar), 85.1 (C-1), 84.7, 82.4, 80.2, 73.9, 73.1, 67.8 ($CH_{2Fmoc}$), 48.3 ($CH_{Fmoc}$), 43.3 ($CH_2$) ppm. HRMS: calcd. for $C_{29}H_{27}NO_7Na$: 524.1680; found *m*/*z* 524.1660.

**Oligonucleotide Synthesis:** Oligonucleotides were synthesized under standard phosphoramidite coupling conditions on a 0.2 μmol scale with polystyrene as the solid support and without use of the final detritylation step in order to leave the 5′-end DMT-protected. The stepwise coupling efficiencies for phosphoramidite **10** were 95%

  

with 15 min coupling time and with 1*H*-tetrazole as catalyst. The coupling yield for unmodified deoxynucleoside phosphoramidites (with 2 min standard coupling time) was >99% with 1*H*-tetrazole as activator. After completion of oligomerization the support was transferred from the reaction column to an Eppendorf tube and treated with piperidine in DMF (20%, 1 mL) for 20 min. The supernatant was removed by syringe (small needle), and the polystyrene was washed with acetonitrile (4×1 mL). A mixture of pyrene-1-carboxylic acid, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (3.7 mg, 9.5 μmol), and diisopropylethylamine (10 μL, 5.7 μmol) in DMF (1 mL) was added to the Eppendorf tube containing the polystyrene, which was then vortexed gently for 45 min. The supernatant was removed by syringe (small needle) and the polystyrene was washed with DMF (2×1 mL) and MeOH (2×1 mL). The oligonucleotide was released from the solid support by treatment with saturated aqueous ammonia for 14 h at 55 °C, which also cleaved off the protecting groups on the nucleobases. The 5′-*O*-DMT-protected oligonucleotides were subsequently purified (minimum 80% purity) on a Waters Prep LC 4000 system fitted with a Xterra MS C18-column (10 μm, 300 mm×7.8 mm) with use of an isocratic hold of 100% A-buffer for 5 min followed by a linear gradient to 55% B-buffer over 75 min at a flow rate of 1.0 mL min$^{-1}$ (A-buffer: 95% 0.1 M $NH_4HCO_3$, 5% $CH_3CN$; B-buffer: 25% 0.1 M $NH_4HCO_3$, 75% $CH_3CN$). The DMT group was cleaved off by treatment with AcOH (80%, 100 μL) for 20 min followed by addition of sodium acetate (3 M, 50 μL), water (100 μL), and ethanol (600 μL). The solution was cooled to –18 °C for 1 hour, which caused the oligonucleotide to precipitate. The oligonucleotide was isolated after centrifugation at 5 °C by decanting of the supernatant.

The compositions of the synthesized ONs were confirmed by MALDI-MS analysis and their purities by analytical ion-exchange HPLC. MALDI-MS: **ON2** [M – H]$^-$ 2903.5 (calcd. 2895.1); **ON5** [M – H]$^-$ 2622.8 (calcd. 2619.8); **ON6** [M – H]$^-$ 2687.0 (calcd. 2686.9); **ON9** [M – H]$^-$ 2825.4 (calcd. 2824.0); **ON10** [M – H]$^-$ 2544.9 (calcd. 2548.6); **ON11** [M – H]$^-$ 2613.9 (calcd. 2615.8); **ON14** [M – H]$^-$ 4385.3 (calcd. 4386.1); **ON15** [M – H]$^-$ 4257.9 (calcd. 4257.0); **ON16** [M – H]$^-$ 4955.5 (calcd. 4956.7); **ON17** [M – H]$^-$3681.6 (calcd. 3686.0).

**Thermal Denaturation Studies:** The thermal denaturation experiments were performed with a Perkin–Elmer UV/Vis lambda 20 spectrometer fitted with a PTP-6 (Peltier Temperature Programmer) device in 1.0 mL cuvettes containing 1.0 μM of each ON in a phosphate buffer. Concentrations of ONs were calculated from the absorbance at 260 nm and the calculated single-strand extinction coefficients based on a nearest neighbor model[47] with 22.4 OD260/μmol as the extinction coefficients for pyrene. The buffer consisted of 100 mM sodium chloride, 10 mM sodium phosphate and 0.1 mM EDTA at pH 7.0.

**Buffer A: Buffer A** was prepared by mixing appropriate volumes of **buffer B** (200 mM sodium chloride, 20 mM $NaH_2PO_4$ and 0.2 mM EDTA) and **buffer C** (200 mM sodium chloride, 10 mM $Na_2HPO_4$ and 0.2 mM EDTA) until a pH value of 7.0 was obtained (using a pH-meter for determination of the pH value). A sample was generally prepared in the following way: the two complementary strands (dissolved in distilled $H_2O$) were added to **buffer A** (500 μL). Distilled $H_2O$ was added to make a total volume of 1000 μL. The samples were heated to 70 °C and cooled to 5 °C before the experiment was initiated with a ramp of 1 °C min$^{-1}$. The thermal denaturation temperature $T_m$ value was determined as the local maximum of the first derivative of the melting curve ($A_{260}$ vs. temperature).

**Fluorescence Experiments:** Steady-state fluorescence emission spectra (360–600 nm) were obtained on a Perkin–Elmer LS 55 luminescence spectrometer fitted with a Peltier temperature controller in quartz cuvettes with a path length of 1.0 cm and a concentration of strands in $T_m$ buffer of 0.15 μM. Spectra were recorded at 20 °C with an average of 5 scans with use of an excitation wavelength of 340 nm, excitation slit of 4.0 nm, emission slit of 2.5 nm, and scan speed of 120 nm min$^{-1}$.

**Supporting Information** (for details see the footnote on the first page of this article): Copies of $^{13}C$ NMR spectra of compounds **2**–**12**.

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[1] A. A. Koshkin, S. K. Singh, P. Nielsen, V. K. Rajwanshi, R. Kumar, M. Meldgaard, C. E. Olsen, J. Wengel, *Tetrahedron* **1998**, *54*, 3607–3630.
[2] A. A. Koshkin, P. Nielsen, M. Meldgaard, V. K. Rajwanshi, S. K. Singh, J. Wengel, *J. Am. Chem. Soc.* **1998**, *120*, 13252–13253.
[3] S. Obika, D. Nanbu, Y. Hari, J.-I. Andoh, K.-I. Morio, T. Doi, T. Imanishi, *Tetrahedron Lett.* **1998**, *39*, 5401–5404.
[4] R. K. Altman, I. Schwope, D. A. Sarracino, C. N. Tetzlaff, C. F. Bleczinski, C. Richert, *J. Comb. Chem.* **1999**, *1*, 493–508.
[5] B. R. Babu, A. K. Prasad, S. Trikha, N. Thorup, V. S. Parmar, J. Wengel, *J. Chem. Soc., Perk. Trans. 1* **2002**, 2509–2519.
[6] R. X. F. Ren, N. C. Chaudhuri, P. L. Paris, S. Rumney IV, E. T. Kool, *J. Am. Chem. Soc.* **1996**, *118*, 7671–7678.
[7] T. J. Matray, E. T. Kool, *J. Am. Chem. Soc.* **1998**, *120*, 6191–6192.
[8] S. Smirnov, T. J. Matray, E. T. Kool, C. de los Santos, *Nucleic Acids Res.* **2002**, *30*, 5561–5569.
[9] T. K. Chakraborty, S. Ghosh, S. Jayaprakash, *Curr. Med. Chem.* **2002**, *9*, 421–435.
[10] F. Schweizer, *Angew. Chem. Int. Ed.* **2002**, *41*, 230–253.
[11] S. A. W. Gruner, E. Locardi, E. Lohof, H. Kessler, *Chem. Rev.* **2002**, *102*, 491–514.
[12] A. Schrey, F. Osterkamp, A. Straudi, C. Rickert, H. Wagner, U. Koert, B. Herrschaft, K. Harms, *Eur. J. Org. Chem.* **1999**, 2977–2990.
[13] R. M. van Well, M. E. A. Meijer, H. S. Overkleeft, J. H. van Boom, G. A. van der Marel, M. Overhand, *Tetrahedron* **2003**, *59*, 2423–2434.
[14] J. Katajisto, H. Lönnberg, *Eur. J. Org. Chem.* **2005**, 3518–3525.
[15] M. Raunkjær, F. El Oualid, G. A. van der Marel, H. S. Overkleeft, M. Overhand, *Org. Lett.* **2004**, *6*, 3167–3170.
[16] R. M. van Well, L. Marinelli, K. Erkelens, G. A. van der Marel, A. Lavecchia, H. S. Overkleeft, J. H. van Boom, H. Kessler, M. Overhand, *Eur. J. Org. Chem.* **2003**, 2303–2313.
[17] E. Lohof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H.-J. Wester, M. Schwaiger, G. Hölzemann, S. L. Goodman, H. Kessler, *Angew. Chem. Int. Ed.* **2000**, *39*, 2761–2764.
[18] D. D. Long, N. L. Hungerford, M. D. Smith, D. E. A. Brittain, D. G. Marquess, T. D. W. Claridge, G. W. J. Fleet, *Tetrahedron Lett.* **1999**, *40*, 2195–2198.
[19] T. D. W. Claridge, D. D. Long, C. M. Baker, B. Odell, G. H. Grant, A. A. Edwards, G. E. Tranter, G. W. J. Fleet, M. D. Smith, *J. Org. Chem.* **2005**, *70*, 2082–2090.
[20] E. Locardi, M. Stöckle, S. Gruner, H. Kessler, *J. Am. Chem. Soc.* **2001**, *123*, 8189–8196.

  

[21] M. Stöckle, G. Voll, R. Günther, E. Lohof, E. Locardi, S. Gruner, H. Kessler, *Org. Lett.* **2002**, *4*, 2501–2504; Correction: M. Stöckle, G. Voll, R. Günther, E. Lohof, E. Locardi, S. Gruner, H. Kessler, *Org. Lett.* **2002**, *4*, 3169.
[22] A. A. Koshkin, J. Fensholdt, H. M. Pfundheller, C. Lomholdt, *J. Org. Chem.* **2001**, *66*, 8504–8512.
[23] R. R. Schmidt, M. Hoffmann, *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 417–418.
[24] R. R. Schmidt, J. Michel, *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 763–764.
[25] R. R. Schmidt, M. Stumpp, J. Michel, *Tetrahedron Lett.* **1982**, *23*, 405–408.
[26] C. Rosenbohm, S. M. Christensen, M. D. Sørensen, D. S. Pedersen, L. E. Larsen, J. Wengel, T. Koch, *Org. Biomol. Chem.* **2003**, *1*, 655–663.
[27] M. Raunkjær, C. E. Olsen, J. Wengel, *J. Chem. Soc., Perkin Trans. 1* **1999**, 2543–2551.
[28] M. Raunkjær, K. F. Haselmann, J. Wengel, *J. Carbohydr. Chem.* **2005**, *24*, 475–502.
[29] D.-H. Kim, H.-S. Rho, J. W. You, J. C. Lee, *Tetrahedron Lett.* **2002**, *43*, 277–279.
[30] J. B. Epp, T. S. Widlanski, *J. Org. Chem.* **1999**, *64*, 293–295.
[31] A. De Mico, T. Margarita, L. Parlanti, A. Vescovi, G. Piancatelli, *J. Org. Chem.* **1997**, *62*, 6974–6977.
[32] B. Venugopalan, C. P. Bapat, P. J. Karnik, D. K. Chatterjee, N. Iyer, D. Lepcha, *J. Med. Chem.* **1995**, *38*, 1922–1927.
[33] T. Bryld, T. Højland, J. Wengel, *Chem. Commun.* **2004**, 1064–1065.
[34] D. Loakes, *Nucleic Acids Res.* **2001**, *29*, 4039–4043.
[35] R. Nichols, P. C. Andrews, P. Zhang, D. E. Bergstrom, *Nature* **1994**, *369*, 492–493.
[36] D. Loakes, D. M. Brown, S. Linde, F. Hill, *Nucleic Acids Res.* **1995**, *23*, 2361–2366.
[37] J. S. Oliver, K. A. Parker, J. W. Suggs, *Org. Lett.* **2001**, *3*, 1977–1980.
[38] D. Loakes, D. M. Brown, S. Linde, F. Hill, *Nucleic Acids Res.* **1995**, *23*, 2361–2366.
[39] M. Berger, Y. Wu, A. K. Ogawa, D. L. McMinn, P. G. Schultz, F. E. Romesberg, *Nucleic Acids Res.* **2000**, *28*, 2911–2914.
[40] F. Seela, H. Debelak, *Nucleic Acids Res.* **2000**, *28*, 3224–3232.
[41] T. J. Matray, E. T. Kool, *Nature* **1999**, *399*, 704–708.
[42] The model structure based on geometry optimization indicates structural compatibility of intercalation of monomer **Z** opposite to an abasic site residue.
[43] L. Margulis, P. F. Pluzhnikov, B. Mao, V. A. Kuzmin, Y. J. Chang, T. W. Scott, N. E. Geacintov, *Chem. Phys. Lett.* **1991**, *187*, 597.
[44] V. V. Shafirovich, S. H. Courtney, N. Q. Ya, N. E. Geacintov, *J. Am. Chem. Soc.* **1995**, *117*, 4920–4929.
[45] C. Brotschi, G. Mathis, C. J. Leumann, *Chem. Eur. J.* **2005**, *11*, 1911–1923.
[46] F. M. Winnik, *Chem. Rev.* **1993**, *93*, 587–614.
[47] G. D. Fasman (Ed.), *Handbook of Biochemistry and Molecular Biology: Nucleic Acids*, 2nd ed., **1985**, vol. 1, p. 589, CRC Press, Boca Raton, FL.

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