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A fluorescence-quencher pair for DNA hybridization studies based on hydrophobic base surrogates

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Abstract—The synthesis of the novel, fluorescent 3-aminobiphenyl-C-nucleoside **M** as well as the corresponding building block for oligodeoxynucleotide synthesis is described. **M** was incorporated into oligodeoxynucleotides via standard phosphoramidite chemistry and the thermal stabilities of duplexes with one and three consecutive **M**–**M**, **M**–**P**, and **M**–**O** pairs, where **P** denotes an unmodified biphenyl C-nucleoside and **O** a 3,5-dinitrobiphenyl-C-nucleoside, were determined. It was found that duplexes containing three consecutive **M**–**O** pairs were the most stable in the series, notably more stable than a duplex with one additional natural G–C pair instead of the modified residues. Furthermore it was found that the fluorescence of **M** is efficiently quenched in a duplex when placed opposite to the dinitrophenyl unit **O**. Thus **M** and **O** constitute a novel fluorophore/quencher pair that is orthogonal to natural base pairs in its recognition properties enabling its use as highly specific tags with reporting properties.

1. Introduction

Fluorescence spectroscopy has lately become a method of choice not only for studying structure and dynamics of nucleic acids down to the single molecule level,[1] but also in various commercialized applications in biotechnology, e.g., DNA sequencing, array hybridization, and real time PCR applications, to name only a few of them. Since natural nucleic acids are non-fluorescent,[2] fluorescence has to be brought about by chemical modification with fluorescent dyes. Alternatively, oligonucleotides can be made fluorescent by replacing natural bases with fluorescent base analogues. The most prominent analogues used in the context outlined above are 2-aminopurine,[3] ethenoadenine,[4] and pyrrolocytosine.[5]

Of special interest were also recent findings with oligonucleotides containing aromatic residues instead of the natural bases. Such constructs are of potential value as orthogonal base pairs for the extension of the genetic alphabet,[6] as tools for probing the enzymatic processivity by polymerases,[7] as tools for DNA damage detection,[8] and as variable color fluorescent tags.[9]

In our own investigations into oligonucleotides with aromatic, hydrophobic residues as base replacements we recently found that biphenyl (**P**) modified oligonucleotides (Fig. 1) readily form stable duplexes via self-recognition of the aromatic units.[10–12] Stability is most likely brought about by interstrand intercalation of two residues in opposite position (Fig. 1). We found that up to seven of such aromatic pairs can be introduced into the center of a duplex with increasing thermal stability per added pair. This interstrand

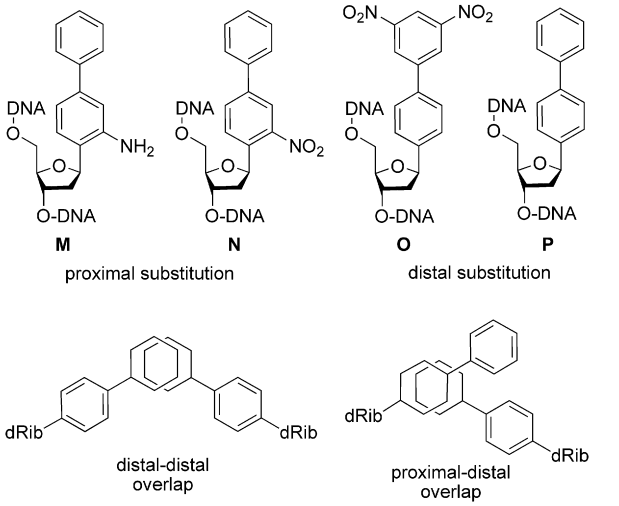

Figure 1. Top: Chemical structures of the biphenyl-C-nucleotides synthesized and used in this study; bottom: two possible, idealized recognition motifs of the biphenyl units within a DNA duplex via partial interstrand stacking interactions.

Keywords: Biphenyl DNA; Fluorescence quenching; DNA analogues; Hydrophobic base pairs.
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recognition motif is not restricted to biphenyl-C-nucleosides but was also reported for non-nucleosidic phenanthrenes and pyrenes, for which interesting excimer formation was observed.[13,14]

The biphenyl scaffold is ideally suited for further chemical modification with functional groups that change its electronic and spectroscopic properties without interfering with its recognition properties. Biphenyl itself is fluorescent but its emission maximum at 318 nm[15] and its fluorescence intensity is far from ideal for applications in biotechnology. Donor-substituted biphenyls show generally red-shifted emission wavelengths and equal to enhanced fluorescence intensities while acceptor-substituted biphenyls, for example, nitrobiphenyls are non-fluorescent and can therefore act as quenchers. The goal of the present study was to explore whether 3-aminobiphenyl-C-nucleoside **N** which displays an emission maximum at 410 nm[15] and 3′,5′-dinitrobiphenyl-C-nucleoside **O** (Fig. 1) could act as such a fluorescence-quencher pair.

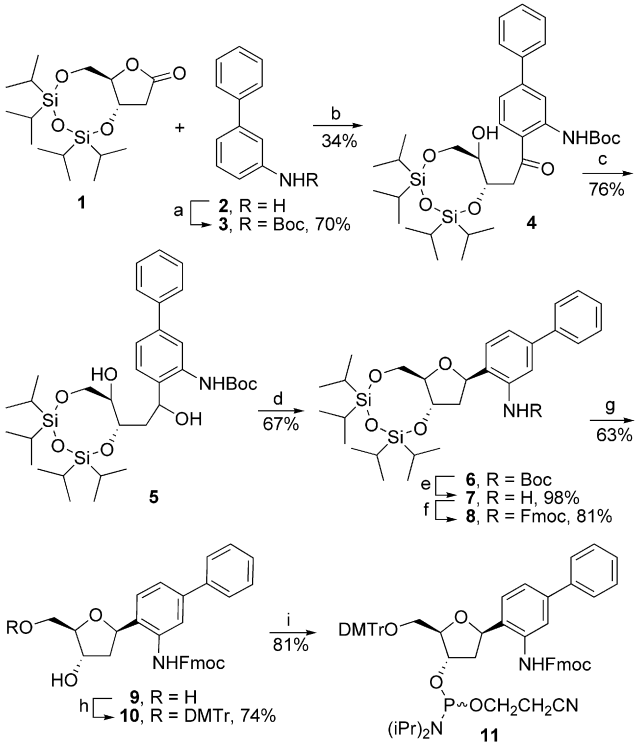
2. Results and discussion

2.1. Synthesis of biaryl-C-nucleosides

The syntheses of the building blocks and of corresponding modified oligodeoxynucleotides with biphenyl-C-nucleosides **O** and **P** were recently published.[12,16] For nucleosides **M** and **N**, carrying an acceptor and a donor group in the proximal phenyl ring, a de novo synthesis had to be devised.

Lithiation of arylhalides followed by nucleophilic addition to lactone derivatives is a well-established pathway in C-nucleoside synthesis.[17,18] However, in the present case where the position neighboring the site of substitution of the biphenyl unit is amino-substituted, direct lithiation via ortho-metalation seemed to be a shorter and better alternative. Especially *tert*-butylcarbamate (Boc) protected aromatic amines are well known for directing metalation into the *ortho* positon.[19,20] Our synthesis therefore started with the Boc-protected aminobiphenyl **3** that was obtained from the known 3-aminobiphenyl **2**[21] by standard methods (Scheme 1).

Ortholithiation of **3** with *tert*-BuLi followed by coupling to the TIPS-protected 2′-deoxy-D-ribono-lactone **1**[22] yielded hydroxy ketone **4** in reasonable yield. No products arising from the nucleophilic attack of the alternative, sterically more hindered *ortho* position could be isolated. Interestingly the ketone and not the corresponding hemiacetal was the predominant product of this reaction, as could readily be determined by ^{13}C NMR. This is most likely due to conjugative stabilization of this keto-function by the Boc-protected amino group (vinologous imide). Classical reduction to the C-nucleoside **6** directly with Et_3SiH and $BF_3\cdot Et_2O$ was therefore expected to be difficult and indeed did not take place. Therefore the method of Reese et al.[23] was chosen for ring closure. For this, compound **4** was reduced with L-Selectride to the corresponding mixture of diastereomeric diols **5** and cyclized under *Mitsunobu* conditions to afford C-nucleoside **6** in a β:α anomeric mixture of ca. 5:1. The β-configuration at the anomeric center was unambiguously assigned by ^1H NMR-NOE-spectroscopy (see Section 4).

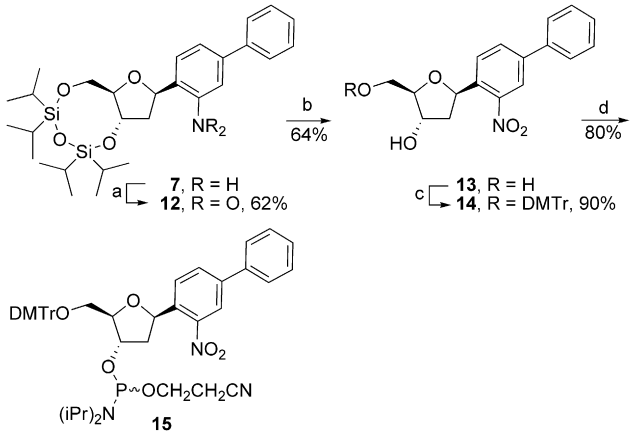


Scheme 1. Reagents and conditions: (a) di-*tert*-butyldicarbonate (1.1 equiv), THF, rt, 15 h; (b) **3** (1 equiv), *t*-BuLi (2.2 equiv), THF, −78 °C to −20 °C (3 h), then **1** (0.6 equiv) in THF, −78 °C, 1.5 h; (c) L-Selectride (1.5 equiv), THF, −78 °C to rt, 30 °C; (d) DIAD (2.5 equiv), PPh_3 (2.5 equiv), THF, 0 °C, 3 h; (e) $SnCl_4$ (4 equiv), EtOAc, rt, 3 h; (f) 9-fluorenylmethylchloroformate (2.2 equiv), *i*-Pr_2NEt (2.2 equiv), CH_2Cl_2, rt, 21 h; (g) $(HF_3)\cdot NEt_3$ (10 equiv), THF, rt, 14 h; (h) 4,4′-dimethoxytrityl (DMTr) chloride (1.2 equiv), pyridine, rt, 4 h; (i) (*i*-$Pr)_2NP(Cl)OCH_2CH_2CN$ (1.5 equiv), *i*-Pr_2NEt (3 equiv), THF, rt, 4 h.

Selective Boc deprotection with $SnCl_4$ in EtOAc[24] followed by reprotection of the amine with 9-fluorenylmethylchloroformate (Fmoc-Cl) under basic conditions (*i*-Pr_2NEt, CH_2Cl_2) yielded Fmoc-protected nucleoside **8** in good yield. Finally, cleavage of the TIPS protection group under mild conditions ($(HF)_3\cdot NEt_3$ in THF) afforded the Fmoc-protected C-nucleoside **9** in acceptable yield. The β-configuration at the pseudoanomeric center in **9** was again confirmed by ^1H NMR-NOE experiments to exclude epimerization during protecting group manipulations. The C-nucleoside **9** was subsequently converted into the corresponding 4,4′-dimethoxytrityl (DMTr)-protected phosphoramidite building block **11** by treatment with 4,4′-dimethoxytritylchloride (DMTrCl) in pyridine (→**10**) followed by phosphitylation in CH_2Cl_2. Phosphoramidite **11** was obtained from **2** in an overall yield of 4%.

With intermediate **7** in hand we also prepared the corresponding *o*-nitrobiphenyl building block **15** (Scheme 2). For this the aromatic amino function in **7** was selectively oxidized with dimethyldioxirane[25] in acetone to give the TIPS-protected nitro-biphenyl-C-nucleoside **12** in 62% yield. Further transformations to the phosphoramidite building block **15** are in exact analogy to those described for the 3-aminobiphenyl-C-nucleoside-phosphoramidite **11**. Proof for β-configuration was again obtained on the level of the sugar deprotected C-nucleoside **13** by ^1H NMR-NOE

experiments (see Section 4). The overall yield for **15** starting from 3-aminobiphenyl **2** was 3%.



Scheme 2. Reagents and conditions: (a) dimethyldioxirane (8.2 equiv), acetone, rt, 3 h; (b) $(HF_3)\cdot NEt_3$ (10 equiv), THF, rt, 17 h; (c) 4,4′-dimethoxytrityl (DMTr) chloride (1.2 equiv), pyridine, rt, 4 h; (d) $(i\text{-Pr})_2NP(Cl)OCH_2CH_2CN$ (1.5 equiv), $i\text{-Pr}_2NEt$ (3 equiv), THF, rt, 4 h.

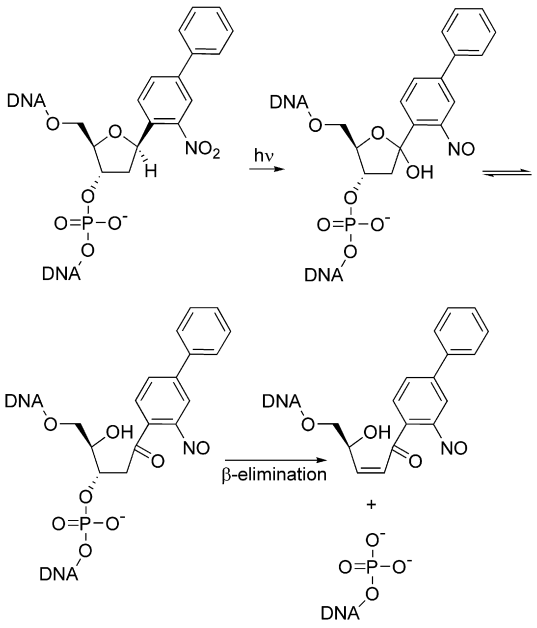
2.2. Synthesis of modified oligodeoxynucleotides

Incorporation of **11** and **15** into oligodeoxynucleotides was performed via standard phosphoramidite chemistry on a DNA synthesizer. The coupling time for the modified building blocks was extended to 6 min, and ethyl-thio-1*H*-tetrazole was used as the activator.[26] Typical coupling yields for the modified building blocks, as deduced from the trityl assay, were in the same range as for non-modified building blocks (>98%). The oligonucleotides were detached from the solid support and deprotected in aqueous ammonia (25%) during 15 h at 55 °C. The oligodeoxynucleotides were purified by RP-HPLC and their structural integrity was verified by ESIMS. (Table 2, Section 4).

2.3. Pairing properties of modified oligodeoxynucleotides

Expectedly, oligodeoxynucleotides containing the *o*-nitrobiphenyl unit **N** were unstable under UV light and led to strand scission at the site of modification, leaving behind two distinct end-phosphorylated fragments as determined by HPLC and ESIMS (data not shown). Strand cleavage most likely arises via the known photochemistry of *o*-nitrobenzyl units[27] via oxidation of the pseudoanomeric center followed by β-elimination of the oligonucleotide fragments (Scheme 3). This C-nucleoside is therefore not suitable for UV-spectroscopic applications but can be used to photolytically cleave oligodeoxynucleotides at predefined positions upon UV irradiation. For this reason the photolytically stable dinitro-C-nucleoside **O** was used as a quencher in the following hybridization experiments.

T_m data were measured for the duplexes indicated in Table 1. Inspection of the data leads to the following general observations. Compared to a natural A–T or G–C base pair, incorporation of a single biphenyl-C-nucleoside pair destabilizes a duplex irrespective of the presence or absence of the amino group. This was expected on the basis of the recognition



Scheme 3. Mechanism of photochemical strand cleavage at *o*-nitrophenyl-C-nucleotide units **N**.

properties of **P**.[12] The problem of the lower affinity can be overcome by increasing the number of biphenyl pairs. Indeed, the T_m's in the triply substituted series are generally higher than that of an identical duplex with one additional natural base pair (Table 1). This is in general agreement with the proposed interstrand-intercalative arrangement of the biphenyls in the duplex.[12]

There are differences in the thermal stability of the biphenyl pairs depending on the nature of the substitutents. In the monosubstituted duplexes, the **M**–**M** pair is less stable than the unfunctionalized **P**–**P** pair. In the triply substituted series, pairing of **M** against the other biphenyls **P** and **O** leads to more stable duplexes than if paired against itself. The duplex with three **M**–**O** pairs is the most stable in this series. The sequential arrangement 5′-**M**-**P**-3′ versus 5′-**P**-**M**-3′ has no major influence on the T_m of a corresponding duplex, indicating stacking interactions of similar energy at the stereochemically different junction sites.

A reason for the diminished stability of **M**–**M** pairs compared to **P**–**P** pairs could be its less hydrophobic nature, favoring its solvation in the single stranded state. Alternatively, steric interactions of the amino group with the backbone or neighboring bases of the same strand may have an

Table 1. T_m data of modified duplexes

5′-d(GATGAC**X**GCTAG)-3′ 3′-d(CTACTG**Y**CGATC)-5′		5′-d(GATGAC**X**$_3$GCTAG)-3′ 3′-d(CTACTG**Y**$_3$CGATC)-5′	
X–Y	T_m	**X–Y**	T_m
M–M	40.6	**M–M**	46.5
R–R	42.5[12]	**P–P**	49.9
C–G	51.9[12]	**M–P**	48.8
T–A	47.9[12]	**P–M**	48.3
		M–O	54.7
		O–O	50.6

c(duplex)=1.2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0.

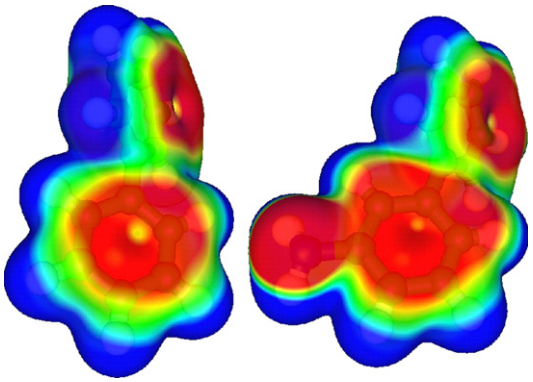


Figure 2. Calculated electron densities of biphenyl (left) and 3-aminobiphenyl (right) in the ground state in the gas phase. Red: high electron density; blue: low electron density. All calculations were performed at the RHF level with the 6-31G(d,p) basis set using *Gaussian 03*.

influence on the stacking interactions and should be considered as well. Finally, π–π stacking in the case of the **M**–**M** pair may be less favored as a consequence of a repulsive contribution of the electron rich π-systems.

A comparison of the structural and electronic properties of the unmodified biphenyl and 3-aminobiphenyl by ab initio calculation in the ground state (Fig. 2) shows that the biarylic axis rotates the two phenyl rings out of plane to approximately the same extent and behaves as a conjugation barrier, leaving the electron densities of the non-substituted phenyl ring in 3-aminobiphenyl on the same level as that in the biphenyl itself. Excluding solvation effects, the difference in stability between **M** and **P** pairs seems thus to be rather of electronic than of structural origin. Given the two most likely, idealized stacking recognition patterns outlined in Figure 1, the observed stability order favors a structural recognition pattern of the proximal/distal type (aminophenyl/phenyl stacking) rather than the distal/distal type (phenyl/phenyl stacking). A qualitative analysis along the same lines may explain the higher stability of the **M**–**O** pair. Here, an attractive dinitrophenyl/phenyl stacking arrangement is expected in the proximal/distal model, which is not the case in the distal/distal model.

2.4. Fluorescence properties of 3-aminobiphenyl containing oligonucleotides

To get insight into the fluorescence properties of the modified oligomers containing the aminobiphenyl unit **M** and the dinitrobiphenyl unit **O** in the paired and unpaired state, emission spectra were recorded from 350 to 450 nm at different temperatures (Fig. 3).

Single strands containing one (data not shown) or three **M**-modifications showed similar spectra with an emission maximum at 408 nm when excited at 237 nm. The incorporated 3-aminobiphenyl units thus show fluorescence behavior similar to that of free 3-aminobiphenyl in water (λ_{max}=410 nm). From this we conclude that no excimeric coupling of the aminobiphenyls occurs in the single strand. Compared to the fluorescence emission properties of aniline (λ_{max}=335 nm),[28] the red-shifted emission maximum of the aminobiphenyl chromophore indicates extended π-conjugation. The fluorescence intensity of the single strand is reduced with increasing temperature in a non-cooperative manner, which excludes self-recognition as fluorescence signal modulating event. A single strand with three dinitrophenyl (**O**) units as a control shows as expected no fluorescence when irradiated at the same wavelength.

A mixture of complementary strands containing three opposing fluorescent **M**-units shows enhanced fluorescence intensity, irrespective of their structural state (duplex vs single strand). Thus, the fluorescence intensity of the aminobiphenyl residues is additive and fairly independent of their structural environment. Also here, slightly reduced

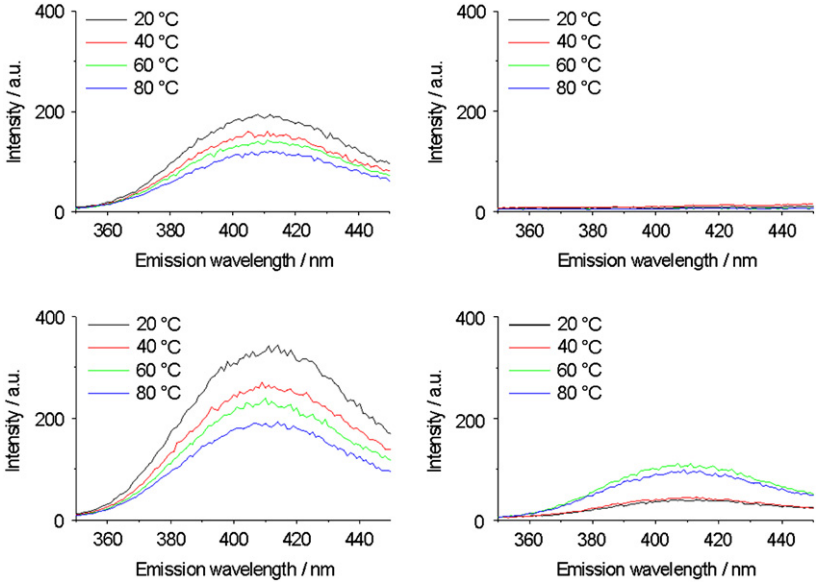


Figure 3. Fluorescence emission spectra at different temperatures of d(GATGAC**M**$_3$GCTAG), top left; d(CTAGC**O**$_3$GTCATC), top right; d(GATGAC**M**$_3$GCTAG)·d(CTAGC**M**$_3$GTCATC), bottom left; d(GATGAC**M**$_3$GCTAG)·d(CTAGC**O**$_3$GTCATC), bottom right. *c*(duplex)=1.2 μM in 3.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris, pH 8.0. Excitation wavelength: 237 nm.

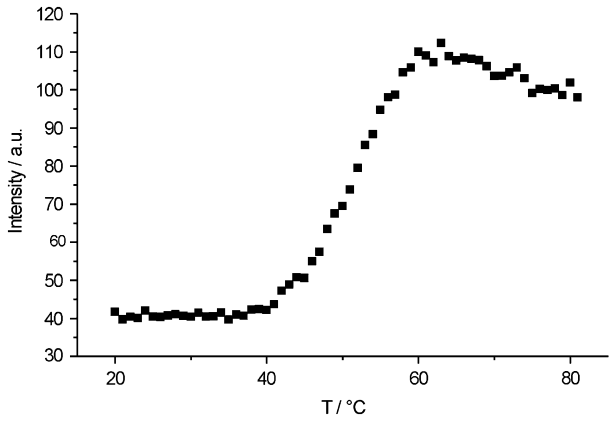


Figure 4. Fluorescence melting curve of the duplex d(GATGAC**M**$_3$GCTAG)·d(CTAGC**O**$_3$GTCATC). *c*(duplex)=1.2 μM in 3.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris, pH 8.0. Excitation wavelength: 237 nm; emission wavelength: 408 nm.

fluorescence intensity at higher temperature was observed. The emission maximum does not change during melting, excluding the formation of excimers also in the duplex. Excimer formation of free aminobiphenyl is not described in the literature but is known for the unsubstituted biphenyl.[29,30]

A duplex with three **M**–**O** pairs shows low fluorescence intensity in the paired state and high intensity in the denatured state. Thus efficient fluorescence quenching of **M** by **O** occurs in the duplexed but not in the single stranded state. The cooperative nature of this change in fluorescence intensity can be seen easily in a corresponding fluorescence melting curve (Fig. 4). The T_m observed (53 °C) is of the same order as that determined from UV-melting curves (Table 1).

2.5. CD spectroscopy

A preliminary investigation on the influence of **M**-residues on the structural properties of the oligonucleotide was performed by CD spectroscopy (Fig. 5). Duplexes with one or three **M**–**M** pairs were measured. The spectra of the duplex with one **M**-residue in each strand show cotton effects that

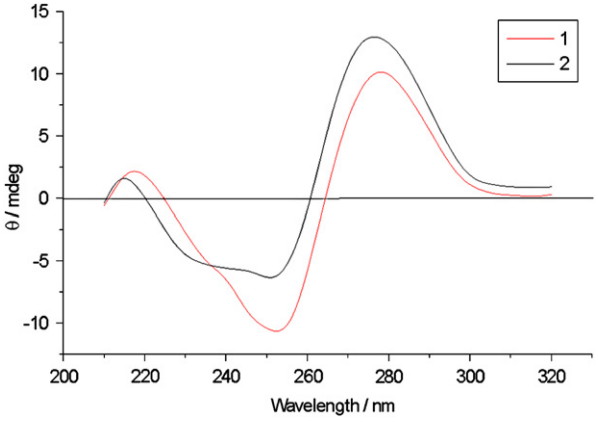


Figure 5. CD spectra of duplex with one **M**–**M** pair (red line) and duplex with three **M**–**M** pairs (black line). *c*(duplex)=3.6 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0, *T*=20 °C.

are typical for B-DNA. The duplex with three **M**–**M** pairs shows a decreased minimum with a shoulder around 240 nm. The positive maximum around 280 nm is slightly increased in intensity. In this case the deviations from the typical B-DNA pattern most likely arise from the increased weight of the spectroscopic properties of the aminobiphenyl chromophore.

3. Conclusions

We presented here the synthesis of the novel fluorescent 3-aminobiphenyl-C-nucleoside **M**. Incorporation of **M** into DNA duplexes was efficiently performed by classical phosphoramidite chemistry. While single incorporations in both strands generally destabilized a duplex, triple incorporations increased its stability to the level of an additional natural base pair. Efficient fluorescence quenching of the fluorescence of **M** is observed in a duplex with the dinitrobiphenyl-C-nucleoside **O** in opposite position. Thus the **M**–**O** system constitutes a novel fluorescence-quencher pair that stabilizes a DNA duplex via interstrand-intercalative stacking interactions and that report presence or absence of a corresponding target by modulation of the fluorescence signal of **M**. This fluorophore/quencher system can be inserted into the center of DNA duplex without destabilizing it. The signal sensitivity can be enhanced by adding further **M**–**O** pairs to the duplex. Furthermore, the **M**–**O** pair is orthogonal to natural base pairs in their recognition properties, enabling its use as highly specific tags with reporting properties.

4. Experimental

4.1. General

All reactions were carried out under argon in glassware, which had been oven dried prior to use. Solvents for reactions (CH_2Cl_2, CH_3CN, Et_2O, THF, toluene) were dried and purified by filtration over alumina or purchased as *crown cap* bottles from *Fluka* (pyridine). Solvents for extractions and flash chromatography (FC) were distilled before use. 2-Cyanoethyldiisopropyl chlorophosphoramidite,[31] dimethyldioxirane,[32] and $TIPDSCl_2$[33] were prepared as described. Silica gel thin layer chromatography (TLC) was performed using *SIL G-25 UV$_{254}$* from *Macherey-Nagel*. Visualization was done either by UV light (254 nm) or by staining solutions (2.1 g cerium(IV) sulfate, 4.2 g phosphomolybdic acid, 12 ml H_2SO_4, 180 ml H_2O or 10 ml *p*-anisaldehyde, 2 ml acetic acid, 10 ml H_2SO_4, 180 ml EtOH). FC was performed with *Silica gel 60* (230–400 mesh) from *Fluka*. ^{1}H NMR spectra were recorded at 300 MHz on a *Bruker AC-300* or on a *Bruker Avance* spectrometer or at 400 MHz on a *Bruker DRX-400* spectrometer. Chemical shifts are reported in parts per million using TMS as an internal standard. Coupling constants *J* are in hertz. Multiplicities are abbreviated as follows: *s*=singlet, *d*=doublet, *t*=triplet, *m*=multiplet. ^{13}C NMR spectra were recorded at 75 MHz on a *Bruker AC-300* or on a *Bruker Avance* spectrometer or at 100 MHz on a *Bruker DRX-400* spectrometer. Chemical shifts are reported in parts per million using TMS as an internal standard. Multiplicities are established by DEPT experiments and are abbreviated as follows: *s*=singlet, *d*=doublet, *t*=triplet,

q=quadruplet. $^{1}H/^{1}H$ and $^{1}H/^{13}C$ correlation experiments (COSY, HMSC) were used for signal assignments. ^{31}P NMR spectra were recorded at 121 MHz on a *Bruker Avance* spectrometer using 85% H_3PO_4 as an external standard. Difference ^{1}H-NOE experiments were recorded on a *Bruker DRX-400* spectrometer. Electron impact (EI) mass spectra were recorded on a *AutoSpeq Q VG* with an ionization energy of 70 eV. Electrospray ionization mass spectra (ESI) were recorded either on a *Fisons Instrument VG Platform* (low resolution) or on an *Applied Biosystems*, *Sciex QSTAR Pulsar* (high resolution). IR-spectra were recorded either on a *Jasco FT/IR-460plus* or on a *Perkin–Elmer Spectrum One*. The absorption bands ν are indicated in cm^{-1}. The intensities are abbreviated as follows: s (strong), m (medium), w (weak).

4.1.1. 3-*N*-(*tert*-Butyloxycarbonyl)aminobiphenyl (3). A solution of 3-aminobiphenyl (2)[21] (3.38 g, 20.0 mmol) and di-*tert*-butyldicarbonate (4.80 g, 22.0 mmol, 1.1 equiv) in dry THF (50 ml) was stirred at rt for 15 h. The mixture was adsorbed on silica gel and purified by FC (hexane/EtOAc, 9:1). The resulting oil was crystallized from hexane to yield **3** as white needles (3.75 g, 13.9 mmol, 70%). TLC (hexane/EtOAc, 9:1): R_f=0.3. ^{1}H NMR (300 MHz, DMSO-d_6, δ (ppm)): 9.43 (*s*); 7.79 (*s*, 1H); 7.60–7.57 (*m*, 2H); 7.49–7.42 (*m*, 3H); 7.39–7.31 (*m*, 2H); 7.24 (*m*, 1H); 1.49 (*s*, 9H). ^{13}C NMR (75 MHz, DMSO-d_6, δ (ppm)): 152.83 (*s*); 140.72 (*s*); 140.33 (*s*); 140.08 (*s*); 129.20 (*d*); 128.91 (2*d*); 127.46 (*d*); 126.60 (2*d*); 120.47 (*d*); 117.19 (*d*); 116.38 (*d*); 79.12 (*s*); 28.13 (*q*). EIMS: 269 (19, M+), 213 (63), 169 (100), 57 (87). HR-EI-MS ($C_{17}H_{19}NO_2$): calcd: 269.141579; found: 269.141480. IR (KBr, cm^{-1}): 3348s, 3055w, 2993m, 2980m, 2934w, 1690s, 1610m, 1600m, 1578w, 1539s, 1500m, 1481s, 1450m, 1405s, 1393m, 1375m, 1366m, 1314m, 1291m, 1278m, 1243s, 1183m, 1152s, 1091m, 1072m, 1047m, 1020m, 996w, 885m, 851m, 800w, 789m, 779w, 761s, 713m, 697m, 662m, 621w, 605w, 521w, 469w.

4.1.2. 1-[3′-*N*-(Butyloxycarbonyl)aminobiphen-4′-yl]-3,5-*O*-tetraisopropyldisilyloxy-4-hydroxypentan-1-one (4). *t*-BuLi (1.7 M in pentane, 5.18 ml, 8.8 mmol, 2.2 equiv) was added to a stirred solution of **3** (1.08 g, 4.00 mmol) in dry THF (10 ml) at −78 °C. The solution was stirred for 1 h before it was allowed to react at −20 °C for another 2 h. The reaction mixture was cooled again to −78 °C and a solution of lactone 1[22] (884.1 mg, 2.36 mmol, 0.59 equiv) in dry THF (10 ml) was added. After stirring for 1.5 h at this temperature the solution was quenched with satd aq NH_4Cl (50 ml) and then extracted with TBME (3×80 ml). The combined organic phases were washed with satd aq NH_4Cl (40 ml), H_2O (40 ml), and brine (40 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by FC (hexane/EtOAc, 20:1→12:1→10:1) yielded the title compound **4** (520.8 mg, 34%) as a yellow foam. TLC (hexane/EtOAc, 9:1): R_f=0.2. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 10.95 (*s*); 8.76 (*d*, *J*=1.9, 1H); 8.00 (*d*, *J*=8.3, 1H); 7.69–7.66 (*m*, 2H); 7.48–7.36 (*m*, 3H); 7.26 (*dd*, J_1=8.3, J_2=1.9, 1H); 4.46–4.39 (*m*, 1H); 4.20 (*dd*, J_1=11.7, J_2=1.1, 1H); 3.84 (*dd*, J_1=11.7, J_2=2.1, 1H); 3.56 (*dd*, J_1=15.4, J_2=3.6, 1H); 3.46–3.39 (*m*, 1H); 3.23 (*dd*, J_1=15.5, J_2=7.1, 1H); 2.46 (*d*, *J*=9.21, 1H); 1.55 (s); 1.09–0.96 (*m*, 28H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 202.58 (*s*); 153.14 (*s*); 147.25 (*s*); 142.19 (*s*); 139.76 (*s*); 131.96 (*d*); 128.82 (2*d*); 128.39 (*d*); 127.42 (2*d*); 120.68 (*s*); 119.55 (*d*); 117.58 (*d*); 80.41 (*s*); 75.30 (*d*); 68.23 (*d*); 62.05, 46.03 (2*t*); 28.34 (*q*); 17.54, 17.39, 17.34, 17.31, 17.23, 17.13 (6*q*); 13.38, 12.89, 12.69, 12.60 (4*d*). HR-ESI$^+$-MS ($C_{34}H_{53}NO_7Si_2Na$, [M+Na]$^+$): calcd: 666.3258; found: 666.3243. IR (neat, cm^{-1}): 2944m, 2867m, 1730m, 1651m, 1612m, 1561m, 1524m, 1495m, 1454m, 1414m, 1367m, 1231m, 1150s, 1084m, 1047s, 1026s, 991m, 958m, 884m, 862m, 759m, 694s.

4.1.3. 1-[3′-*N*-(Butyloxycarbonyl)aminobiphen-4′-yl]-3,5-*O*-tetraisopropyldisilyloxy-1,4-dihydroxypentan (5). L-Selectride (1 M in THF, 2.76 ml, 2.76 mmol, 1.5 equiv) was added dropwise to a stirred solution of **4** (1.18 g, 1.84 mmol) in dry THF (12 ml) at −78 °C over 10 min. The solution was stirred for 5 min at this temperature and then 30 min at rt. The mixture was quenched with satd aq $NaHCO_3$ (40 ml) and then extracted with CH_2Cl_2 (3× 80 ml). The combined organic phases were washed with satd aq $NaHCO_3$ (50 ml), H_2O (50 ml), and brine (50 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by FC (hexane/EtOAc, 10:2) yielded the title compound **5** (906.6 mg, 1.40 mmol, 76%) as a white foam. TLC (hexane/EtOAc, 10:2): R_f=0.2. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.59 (*s*, 1H); 8.33 (*s*, 1H); 7.63–7.60 (*m*, 2H); 7.43–7.38 (*m*, 2H); 7.34–7.31 (*m*, 1H); 7.23–7.20 (*m*, 2H); 5.27–5.23 (*m*, 1H); 4.24 (*dd*, J_1=11.7, J_2=0.9, 1H); 4.12–4.07 (*m*, 1H); 3.91 (*dd*, J_1=11.8, J_2=2.0, 1H); 3.79 (*d*, *J*=9.61, 1H); 2.49–2.39 (*m*, 1H); 2.22–2.15 (*m*, 1H); 1.53 (*s*, 9H); 1.18–1.03 (*m*, 28H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 153.23 (*s*); 141.24, 140.85, 137.77, 129.59 (4*s*); 128.61, 127.59, 127.24, 121.43, 119.45 (5*d*); 79.92 (*s*); 72.49, 72.05, 70.42 (3*d*); 62.47, 39.03 (2*t*); 28.43 (*q*); 17.69, 17.55, 17.32, 17.27, 17.26, 17.21 (6*q*); 13.35, 13.31, 12.65, 12.55 (4*d*). HR-ESI$^+$-MS ($C_{34}H_{55}NO_7Si_2Na$, [M+Na]$^+$): calcd: 668.3414; found: 668.3421. IR (neat, cm^{-1}): 3336w, 2944w, 2867w, 2359w, 1727m, 1587w, 1570w, 1532w, 1494w, 1464m, 1415m, 1389w, 1366m, 1241m, 1159m, 1048s, 1026s, 918w, 885m, 826m, 761m, 695s.

4.1.4. 3-*N*-(Butyloxycarbonyl)amino-4-(2′-deoxy-3′,5′-*O*-tetraisopropyldisilyloxy-β-D-ribofuranosyl)biphenyl (6). DIAD (678 µl, 3.50 mmol, 2.5 equiv) was added dropwise to a stirred solution of **5** (903 mg, 1.40 mmol) and PPh_3 (918 mg, 3.50 mmol, 2.5 equiv) in dry THF (25 ml) at 0 °C. This solution was stirred for 3 h at this temperature. A 0.5 M solution of I_2 in CH_2Cl_2 was added dropwise until the iodine coloration just persisted. After concentration under reduced pressure, the residue was taken up in satd aq $NaHCO_3$ (40 ml). The mixture was extracted with CH_2Cl_2 (3×100 ml). The combined organic phases were washed with satd aq $NaHCO_3$ (50 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by FC (hexane/EtOAc, 100:1→9:1) yielded the title compound **6** (585.5 mg, 0.93 mmol, 67%, β/α~5:1) as a white foam. TLC (2% EtOAc in hexane): R_f=0.1. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.25 (*s*, 1H); 8.01 (*s*, 1H); 7.63–7.60 (*m*, 2H); 7.43–7.38 (*m*, 2H); 7.35–7.29 (*m*, 1H); 7.24–7.22 (*m*, 2H); 5.16 (*dd*, J_1=9.8, J_2=6.1, 1H); 4.59–4.54 (*m*, 1H); 4.19 (*dd*, J_1=11.1, J_2=3.6, 1H); 4.04–3.98 (*m*, 1H); 3.84 (*dd*, J_1=10.9, J_2=9.6, 1H); 2.46–2.35 (*m*, 1H); 2.28 (*ddd*, J_1=13.4, J_2=6.1, J_3=2.6, 1H); 1.53 (*s*, 9H); 1.12–1.02 (*m*, 28H). ^{1}H NMR-NOE (400 MHz, $CDCl_3$, δ (ppm)): 5.16 (H-C(1′))→4.01 (H-C(4′), 6%), 2.28 (H_α-C(2′), 4%); 4.57 (H-C(3′))→4.01

(H-C(4′), 3%), 2.40 (H_β-C(2′), 6%). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 152.94 (*s*); 141.82, 140.67, 137.69 (3*s*); 128.64, 127.85, 127.37, 127.24, 121.46 (5*d*); 87.43 (*d*); 80.13 (*s*); 79.79, 74.87 (2*d*); 64.60, 41.03 (2*t*); 28.39 (*q*); 17.59, 17.48, 17.43, 17.40, 17.30, 17.12, 17.06, 17.00 (8*q*); 13.50, 13.44, 13.07, 12.62 (4*d*). HR-ESI$^+$-MS ($C_{34}H_{53}NO_6Si_2Na$, $[M+Na]^+$): calcd: 650.3309; found: 650.3300. IR (neat, cm^{-1}): 3357w, 2944m, 2867m, 2360m, 2341w, 1731m, 1587w, 1570m, 1532w, 1494w, 1464m, 1417m, 1389w, 1366m, 1242m, 1156s, 1085m, 1032s, 955m, 919w, 884m, 824m, 759m, 693s, 637m.

4.1.5. 3-Amino-4-(2′-deoxy-3′,5′-*O*-tetraisopropyldisilyloxy-β-D-ribofuranosyl)biphenyl (7). $SnCl_4$ (435 μl, 3.72 mmol, 4 equiv) was added dropwise to a stirred solution of **6** (585 mg, 0.93 mmol) in dry EtOAc (14 ml) at rt. This solution was stirred for 3 h at this temperature. The mixture was quenched with satd aq $NaHCO_3$ (40 ml) and then extracted with TBME (3×100 ml). The combined organic phases were washed with satd aq $NaHCO_3$ (50 ml), H_2O (50 ml), and brine (50 ml), dried over $MgSO_4$, and concentrated in vacuo. Purification by FC (hexane/EtOAc, 10:1, 1% Et_3N) yielded the title compound **7** (483.2 mg, 0.92 mmol, 98%, β/α~4:1) as a slightly yellow oil. TLC (hexane/EtOAc, 10:1): R_f=0.2. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 7.56–7.53 (*m*, 2H); 7.42–7.38 (*m*, 2H); 7.34–7.29 (*m*, 1H); 7.16 (*d*, *J*=7.92, 1H); 6.98–6.90 (*m*, 2H); 5.15 (*dd*, J_1=8.7, J_2=7.0, 1H); 4.64–4.58 (*m*, 1H); 4.40 (*s*, 2H, amine); 4.12 (*dd*, J_1=10.5, J_2=2.4,1H); 3.93–3.83 (*m*, 2H); 2.57–2.41 (*m*, 1H); 2.29 (*ddd*, J_1=12.8, J_2=7.0, J_3=4.1, 1H); 1.13–0.96 (*m*, 28H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 141.87, 141.07 (2*s*); 128.62, 127.96, 127.21, 127.03 (4*d*); 123.71 (*s*); 117.23, 115.31 (2*d*); 86.54, 78.86, 73.32 (3*d*); 63.45, 39.40 (2*t*); 17.61, 17.49, 17.46, 17.40, 17.27, 17.12, 17.10, 17.00 (8*q*); 13.53, 13.37, 13.09, 12.58 (4*d*). HR-ESI$^+$-MS ($C_{29}H_{46}NO_4Si_2$, $[M+H]^+$): calcd: 528.2965; found: 528.2965. IR (neat, cm^{-1}): 3453w, 3361w, 2943m, 2866m, 2361w, 1620w, 1565w, 1487w, 1463m, 1419w, 1385w, 1317w, 1285w, 1246w, 1180w, 1117m, 1082m, 1033s, 957m, 918m, 883m, 758s, 693s, 637m.

4.1.6. 3-*N*-(9-Fluorenylmethoxycarbonyl)amino-4-(2′-deoxy-3′,5′-*O*-tetraisopropyldisilyloxy-β-D-ribofuranosyl)biphenyl (8). A mixture of **7** (206 mg, 0.39 mmol), 9-fluorenylmethylchloroformate (222 mg, 0.86 mmol, 2.2 equiv), and *N*-ethyldiisopropylamine (0.147 ml, 0.86 mmol, 2.2 equiv) in dry CH_2Cl_2 (2 ml) was stirred for 21 h at rt. The mixture was quenched with H_2O (20 ml) and then extracted with CH_2Cl_2 (3×80 ml). The combined organic phases were washed with H_2O (30 ml) and brine (30 ml), dried ($MgSO_4$), and concentrated in vacuo. Purification by FC (2% EtOAc in hexane) yielded the title compound **8** (236.3 mg, 0.31 mmol, 81%, β/α~4:1) as a white foam. TLC (hexane/EtOAc, 10:1): R_f=0.6. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.30 (*s*, 1H); 7.80–7.77 (*m*, 2H); 7.62–7.60 (*m*, 4H); 7.44–7.22 (*m*, 10H); 5.21–5.13 (*m*, 1H); 4.58–4.54 (*m*, 1H); 4.52–4.45 (*m*, 2H); 4.31 (*t*, *J*=7.0, 1H); 4.15 (*dd*, J_1=11.0, J_2=3.9, 1H); 4.08–4.02 (*m*, 1H); 3.75 (*dd*, J_1=10.8, J_2=9.9, 1H); 2.38–2.26 (*m*, 2H); 1.15–0.98 (*m*, 28H). ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 153.50, 143.87, 143.77, 141.88, 141.39, 141.36, 140.43, 137.27 (8*s*); 128.71, 127.90, 127.74, 127.50, 127.20, 127.19, 127.10, 125.14, 124.99, 120.08, 120.05, 120.01 (12*d*); 87.82, 83.70, 74.42 (3*d*); 66.84, 64.34 (2*t*); 47.21 (*d*); 40.96 (*t*); 17.63, 17.48, 17.45, 17.40, 17.32, 17.13, 17.09, 17.00 (8*q*); 13.57, 13.51, 13.15, 12.57 (4*d*). HR-ESI$^+$-MS ($C_{44}H_{56}NO_6Si_2$, $[M+H]^+$): calcd: 750.3646; found: 750.3656. IR (neat, cm^{-1}): 3346w, 2943m, 2866m, 2359w, 1736m, 1587w, 1570m, 1536m, 1496w, 1450m, 1417m, 1243m, 1206s, 1135m, 1084m, 1033s, 884m, 824w, 758s, 737s, 693s, 662m, 651m, 642m, 610m.

4.1.7. 3-*N*-(9-Fluorenylmethoxycarbonyl)amino-4-(2′-deoxy-β-D-ribofuranosyl)biphenyl (9). To a mixture of **8** (129 mg, 0.17 mmol) in dry THF (8.5 ml) was added dropwise $(HF)_3\cdot NEt_3$ (277 μl, 1.72 mmol, 10 equiv). After stirring for 14 h at rt, the solution was concentrated in vacuo. Purification by FC (2% MeOH in CH_2Cl_2) yielded the title compound **9** (53.9 mg, 0.11 mmol, 63%, β only) as a colorless foam. TLC (5% MeOH in CH_2Cl_2): R_f=0.5. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.62 (*s*, 1H); 8.28 (*s*, 1H); 7.77 (*d*, *J*=7.3, 2H); 7.62–7.57 (*m*, 4H); 7.42–7.29 (*m*, 7H); 7.20 (*dd*, J_1=7.9, J_2=1.7, 1H); 7.11 (*d*, *J*=8.1, 1H); 5.13 (*dd*, J_1=11.1, J_2=5.5, 1H); 4.72 (*d*, *J*=5.1, 2H); 4.29 (*s*, 1H); 4.25 (*t*, *J*=5.1, 1H); 3.91 (*d*, *J*=2.6, 1H); 3.49 (*s*, 2H); 2.12 (*m*, 1H); 1.97 (*dd*, J_1=13.1, J_2=5.4, 1H). ^{1}H NMR-NOE (400 MHz, $CDCl_3$, δ (ppm)): 5.13 (H-C(1′))→3.91 (H-C(4′), 4%), 1.97 (H_α-C(2′), 5%); 4.29 (H-C(3′))→3.91 (H-C(4′), 2%), 2.12 (H_β-C(2′), 5%). ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 143.86, 143.77, 141.87, 141.43, 141.25, 140.48, 137.39 (7*s*); 128.84, 128.69, 127.84, 127.76, 127.46, 127.42, 127.36, 127.18, 124.74, 124.71, 119.96, 119.91 (12*d*); 87.51, 81.42, 73.37 (3*d*); 65.28, 62.41 (2*t*); 47.36 (*d*); 41.92 (*t*). HR-ESI$^+$-MS ($C_{32}H_{29}NO_5Na$, $[M+Na]^+$): calcd: 530.1943; found: 530.1948. IR (neat, cm^{-1}): 3322w, 2360w, 1701w, 1568w, 1536w, 1449w, 1415m, 1208m, 1044m, 945w, 907m, 759m, 731s, 696m.

4.1.8. 3-*N*-(9-Fluorenylmethoxycarbonyl)amino-4-[(5′-*O*-4,4′-dimethoxytriphenyl)methyl-2′-deoxy-β-D-ribofuranosyl]biphenyl (10). Diol **9** (144 mg, 0.28 mmol) was coevaporated with pyridine (3×2 ml) and then dissolved in pyridine (1.2 ml). DMTrCl was added in portions over 2 h (4×28.8 mg; totally 0.34 mmol, 1.2 equiv). After stirring for another 90 min at rt, the mixture was diluted with EtOAc (100 ml) and washed with satd aq $NaHCO_3$ solution (3×25 ml). The combined aqueous phases were extracted with EtOAc (3×25 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The residue was coevaporated with toluene (3×4 ml). Purification by FC (equilibration with hexane+1% Et_3N, then hexane; elution with hexane/EtOAc, 7:3) yielded the title compound **10** (168.0 mg, 0.21 mmol, 74%) as a colorless foam. TLC (hexane/EtOAc, 7:3): R_f=0.2. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.51 (*s*, 1H); 8.31 (*s*, 1H); 7.76 (*d*, *J*=7.5, 2H); 7.64–7.61 (*m*, 2H); 7.58–7.53 (*m*, 2H); 7.44–7.27 (*m*, 11H); 7.25–7.12 (*m*, 7H); 6.72–6.68 (*m*, 4H); 5.34 (*dd*, J_1=10.4, J_2=5.7, 1H); 4.46–4.42 (*m*, 1H); 4.40–4.29 (*m*, 2H); 4.20–4.16 (*m*, 2H); 3.68 (*d*, *J*=2.8, 6H); 3.42 (*dd*, J_1=9.8, J_2=5.1, 1H); 3.27 (*dd*, J_1=10.0, J_2=5.7, 1H); 2.45–2.35 (*m*, 1H); 2.32–2.25 (*m*, 1H); 1.87 (*s*, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 158.49, 153.68, 144.73, 143.93, 143.84, 141.83, 141.30, 140.45, 137.55, 135.80, 135.78 (11*s*); 130.05, 128.72, 128.14, 127.82, 127.73,

127.51, 127.30, 127.21, 127.13, 126.82, 125.18, 119.98, 113.10, 86.67 (14*d*); 86.38 (*s*); 79.00, 74.05 (2*d*); 67.07, 64.43 (2*t*); 55.12 (*q*); 47.06 (*d*); 40.36 (*t*). HR-ESI$^+$-MS ($C_{53}H_{47}NO_7$, [M+Na]$^+$): calcd: 832.3250; found: 832.3228. IR (neat, cm^{-1}): 3328w, 2929w, 2360w, 1732m, 1606m, 1569m, 1535m, 1507m, 1448m, 1415m, 1301m, 1247s, 1212m, 1175m, 1034s, 949m, 910m, 826m, 759m, 738s, 698s, 647m, 635m, 614m.

4.1.9. 3-*N*-(9-Fluorenylmethoxycarbonyl)amino-4-[(5′-*O*-4,4′-dimethoxytriphenyl)methyl-3′-(*N*,*N*-diisopropylamino-2-cyanoethyl-phosphino)-2′-deoxy-β-D-ribofuranosyl]biphenyl (11). To a solution of **10** (121 mg, 0.15 mmol) in dry THF (4 ml), *N*,*N*-diisopropylethylamine (76.8 µl, 0.45 mmol, 3 equiv) followed by 2-cyanoethyl-diisopropylchlorophosphoramidite (50.3 µl, 0.23 mmol, 1.5 equiv) was added at rt. After stirring for 4 h (TLC control), EtOAc (100 ml) was added and the mixture was washed with satd aq $NaHCO_3$ solution (3×25 ml). The combined aqueous phases were extracted with EtOAc (3×25 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. Purification by FC (equilibration with hexane+1% Et_3N, then hexane; elution with hexane/EtOAc, 7:3) yielded the title compound **11** (123.1 mg, 0.12 mmol, 81%) as a white foam. TLC (silica gel; hexane/EtOAc, 7:3): R_f=0.5. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.68 (*s*, 1H); 8.36 (*s*, 1H); 7.77 (*d*, *J*=7.5, 2H); 7.65–7.53 (*m*, 4H); 7.45–7.29 (*m*, 12H); 7.23–7.16 (*m*, 6H); 6.72–6.67 (*m*, 4H); 5.39–5.32 (*m*, 1H); 4.59–4.41 (*m*, 1H); 4.35–4.26 (*m*, 3H); 4.16 (*t*, *J*=7.8, 1H); 3.91–3.63 (*m*, 10H); 3.35–3.27 (*m*, 2H); 2.67–2.41 (*m*, 4H); 1.32–1.12 (*m*, 12H). ^{31}P NMR (121 MHz, $CDCl_3$, δ (ppm)): 149.15, 148.57. ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 158.44, 158.43, 153.74, 153.70, 144.75, 143.98, 143.85, 141.83, 141.76, 141.28, 140.50, 140.47, 137.72 (13*s*); 130.12, 130.08, 128.72, 128.23, 128.19, 127.76, 127.71, 127.50, 127.28, 127.22, 127.11, 126.78, 126.73, 125.21, 121.91, 119.99 (16*d*); 117.53, 117.45 (2*s*); 113.06, 113.04, 86.42 (3*d*); 86.38, 86.26, 86.21 (3*s*); 86.14, 86.07, 79.22, 78.97, 75.58, 75.40, 75.34, 75.16 (8*d*); 67.17, 64.30, 64.19, 58.49, 58.38, 58.30, 58.19 (7*t*); 55.12 (*q*); 47.03, 43.41, 43.35, 43.29, 43.23 (5*d*); 39.53 (*t*); 24.68, 24.60, 24.57, 24.49 (4*q*); 20.45, 20.38, 20.26, 20.19 (4*t*). ESI$^+$-MS: 1010.4 ([M+H]$^+$). IR (KBr, cm^{-1}): 3435m, 3066w, 2968m, 2932m, 2838w, 2254w, 1735m, 1609m, 1588m, 1571m, 1539m, 1510s, 1479m, 1464m, 1452m, 1416m, 1397m, 1365m, 1303m, 1251s, 1212s, 1180s, 1157m, 1127m, 1077s, 1036s, 1002m, 979m, 891m, 829m, 791w, 761m, 742m, 727m, 700m, 585m, 544w.

4.1.10. 3-Nitro-4-(2′-deoxy-3′,5′-*O*-tetraisopropyldisilyl-oxy-β-D-ribofuranosyl)biphenyl (12). To a solution of **7** (240 mg, 0.45 mmol) in acetone (9.5 ml) was added dimethyldioxirane (0.085 M in acetone, 44 ml, 3.7 mmol, 8.2 equiv). After stirring for 3 h at rt in the dark, the solution was concentrated in vacuo and then coevaporated with toluene (3×4 ml). Purification by FC (2% EtOAc in hexane) yielded the title compound **12** (156.6 mg, 0.28 mmol, 62% α:β∼10:1) as a yellow oil. TLC (2% EtOAc in hexane): R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.26 (*d*, *J*=1.9, 1H); 8.05 (*d*, *J*=8.3, 1H); 7.83 (*dd*, J_1=8.1, J_2=1.9, 1H); 7.60–7.59 (*m*, 2H); 7.51–7.41 (*m*, 3H); 5.66 (*dd*, J_1=8.6, J_2=5.0, 1H); 4.47 (*dd*, J_1=15.3, J_2=7.7, 1H); 4.12 (*d*, J_1=3.39, 2H); 3.87–3.82 (*m*, 1H); 2.80–2.70 (*m*, 1H); 2.12 (*ddd*, J_1=12.8, J_2=7.7, J_3=5.1, 1H); 1.12–0.99 (*m*, 28H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 147.32, 141.44, 138.63, 138.56 (4*s*); 132.13, 129.27, 128.57, 128.41, 127.18, 123.04 (6*d*); 85.01, 74.63, 70.40 (3*d*); 61.91, 42.07 (2*t*); 17.70, 17.61, 17.54, 17.35, 17.22, 17.13, 16.98 (7*q*); 13.62, 13.37, 13.07, 12.73 (4*d*). HR-ESI$^+$-MS ($C_{29}H_{44}NO_6Si_2$, [M+H]$^+$): calcd: 558.2707; found: 558.2723. IR (neat, cm^{-1}): 2943m, 2866m, 2361w, 1530m, 1508w, 1464m, 1386w, 1348m, 1245w, 1122m, 1091m, 1065m, 1034s, 957w, 919w, 883s, 851m, 760m, 693s, 607m.

4.1.11. 3-Nitro-4-(2′-deoxy-β-D-ribofuranosyl)biphenyl (13). To a mixture of **12** (393 mg, 0.70 mmol) in dry THF (35.5 ml) was added dropwise $(HF)_3 \cdot NEt_3$ (1.16 ml, 7.04 mmol, 10 equiv). After stirring for 17 h at rt, the solution was concentrated in vacuo. Purification by FC (silica gel; 2% MeOH in CH_2Cl_2) and recrystallization from CH_2Cl_2/hexane yielded the title compound **13** (142.6 mg, 0.45 mmol, 64%, β only) as a white solid. TLC (5% MeOH in CH_2Cl_2): R_f=0.3. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.19 (*s*, 1H); 7.89–7.83 (*m*, 2H); 7.62–7.60 (*m*, 2H); 7.52–7.42 (*m*, 3H); 5.70 (*dd*, J_1=9.4, J_2=6.2, 1H); 4.48–4.44 (*m*, 1H); 4.07–4.04 (*m*, 1H); 3.97–3.84 (*m*, 2H); 2.64 (*ddd*, J_1=13.3, J_2=6.0, J_3=2.5); 2.08–1.98 (*m*, 1H). ^{1}H NMR-NOE (400 MHz, MeOD, δ (ppm)): 5.59 (H-C(1′))→3.97 (H-C(4′), 3%), 2.54 (H_α-C(2′), 5%); 4.33 (H-C(3′))→3.97 (H-C(4′), 3%), 1.93 (H_β-C(2′), 6%). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 148.02, 41.62, 138.29, 136.29 (4*s*, C(4)); 131.88, 129.18 (2*d*); 128.53, 127.90, 127.04, 122.83, 86.79, 76.10, 73.35 (7*d*, C(3″)); 63.35, 43.90 (2*t*). HR-ESI$^+$-MS ($C_{17}H_{17}NO_5$, [M+Na]$^+$): calcd: 338.1004; found: 338.1005. IR (neat, cm^{-1}): 3222w, 2360w, 1531m, 1340s, 1090m, 1052s, 998m, 954m, 893w, 850w, 753s, 697m, 680m, 620w.

4.1.12. 3-Nitro-4-[(5′-*O*-4,4′-dimethoxytriphenyl)methyl-2′-deoxy-β-D-ribofuranosyl]biphenyl (14). Diol **13** (162 mg, 0.51 mmol) was coevaporated with pyridine (3×4 ml) and then dissolved in pyridine (2.2 ml). DMTrCl was added in portions over 2 h (4×52.6 mg; totally 0.62 mmol, 1.2 equiv). After stirring another 90 min at rt, the mixture was diluted with EtOAc (100 ml) and washed with satd aq $NaHCO_3$ solution (3×25 ml). The combined aqueous phases were extracted with EtOAc (3×25 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. The residue was coevaporated with toluene (3×4 ml). Purification by FC (hexane/EtOAc, 8:2, 1% Et_3N) yielded the title compound **14** (284.7 mg, 0.46 mmol, 90%) as a yellow foam. TLC (silica gel; hexane/EtOAc, 7:3): R_f=0.4. ^{1}H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.21 (*d*, *J*=1.9, 1H); 8.03 (*d*, *J*=8.3, 1H); 7.75 (*dd*, J_1=8.1, J_2=1.9, 1H); 7.62–7.58 (*m*, 2H); 7.51–7.46 (*m*, 4H); 7.44–7.40 (*m*, 1H); 7.39–7.35 (*m*, 4H); 7.33–7.27 (*m*, 2H); 7.25–7.19 (*m*, 1H); 6.87–6.82 (*m*, 4H); 5.71 (*dd*, J_1=8.8, J_2=6.3, 1H); 4.46–4.41 (*m*, 1H); 4.10–4.07 (*m*, 1H); 3.79 (*s*, 6H); 3.43 (*d*, *J*=4.5, 2H); 2.64 (*ddd*, J_1=13.2, J_2=6.4, J_3=3.4, 1H); 2.11–2.01 (*m*, 1H); 1.78 (*s*, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ (ppm)): 158.58, 147.81, 144.79, 141.36, 138.39, 137.37, 135.97 (7*s*); 131.91, 130.15, 130.13, 129.14, 128.45, 128.42, 128.23, 127.90, 127.03, 126.90, 122.71, 113.21 (12*d*); 86.43 (*s*); 85.72, 75.89, 73.90 (3*d*); 63.96 (*t*); 55.23 (*q*); 43.49 (*t*). HR-ESI$^+$-MS

($C_{38}H_{35}NO_7$, $[M+Na]^+$): calcd: 640.2311; found: 640.2315. IR (neat, cm^{-1}): 2931w, 2360m, 1733w, 1606w, 1528m, 1506s, 1443m, 1349m, 1299m, 1246s, 1174m, 1031s, 826s, 754s, 724m, 697s, 680s, 647m, 624m, 612s.

4.1.13. 3-Nitro-4-[(5′-*O*-4,4′-dimethoxytriphenyl)methyl-3′-(*N*,*N*-diisopropylamino-2-cyanoethyl-phosphino)-2′-deoxy-β-D-ribofuranosyl]biphenyl (15). To a solution of **14** (285 mg, 0.46 mmol) in dry THF (12 ml) was added at rt *N*,*N*-diisopropylethylamine (236 µl, 1.38 mmol, 3 equiv) followed by 2-cyanoethyldiisopropyl chlorophosphoramidite (151 µl, 0.69 mmol, 1.5 equiv). After stirring for 4 h (TLC control), EtOAc (100 ml) was added and the mixture was washed with satd aq $NaHCO_3$ solution (3×25 ml). The combined aqueous phases were extracted with EtOAc (3×25 ml). The combined organic phases were dried ($MgSO_4$) and concentrated in vacuo. Purification by FC (hexane/EtOAc, 8:2, 1% Et_3N) yielded the title compound **15** (300.0 mg, 0.37 mmol, 80%) as a white foam. TLC (hexane/EtOAc, 8:2): R_f=0.3. ^1H NMR (300 MHz, $CDCl_3$, δ (ppm)): 8.22–8.21 (*m*, 1H); 8.08 (*dd*, J_1=12.4, J_2=8.2, 1H); 7.75 (*dt*, J_1=8.2, J_2=2.2, 1H); 7.61–7.59 (*m*, 2H); 7.51–7.46 (*m*, 4H); 7.43–7.35 (*m*, 5H); 7.32–7.28 (*m*, 2H); 7.24–7.21 (*m*, 1H); 6.86–6.81 (*m*, 4H); 5.70 (*dd*, J_1=9.3, J_2=5.7, 1H); 4.59–4.49 (*m*, 1H); 4.24–4.23 (*m*, 1H); 3.90–3.83 (*m*, 1H); 3.79, 3.78 (2*s*, 6H); 3.68–3.55 (*m*, 3H); 3.50–3.34 (*m*, 2H); 2.83–2.71 (*m*, 1H); 2.66 (*t*, *J*=6.3, 1H); 2.44 (*t*, *J*=6.5, 1H); 2.08–1.97 (*m*, 1H); 1.19–1.07 (*m*, 12H). ^{31}P NMR (121 MHz, $CDCl_3$, δ (ppm)): 148.90, 147.98. ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 158.54, 147.92, 147.87, 144.83, 144.78, 141.35, 141.34, 138.39, 137.32, 137.12, 136.03, 135.98 (12*s*); 131.98, 131.95, 130.24, 130.21, 130.17, 130.15, 129.14, 128.52, 128.44, 128.35, 128.27, 127.84, 127.03, 126.87, 126.83, 122.73, 122.67 (17*d*); 117.68, 117.47 (2*s*); 113.13 (*d*); 86.27 (s); 85.47, 85.44 (2d); 85.42 (s); 76.25, 76.16, 75.43, 75.27, 75.12, 74.95 (6*d*); 63.77, 63.45, 58.56, 58.38, 58.34, 58.15 (6*t*); 55.24, 55.22 (2*q*); 43.42, 43.29, 43.16, 42.88, 42.84 (5*d*); 42.69, 42.65 (2*t*); 24.67, 24.63, 24.60, 24.57, 24.53, 24.50, 24.45 (7*q*); 20.47, 20.41, 20.21, 20.14 (4*t*). ESI^+-MS: 818.4 ($[M+H]^+$). IR (KBr, cm^{-1}): 3439m, 3060w, 3036w, 2968m, 2932m, 2874m, 2838w, 2254w, 2051w, 1609m, 1584w, 1532s, 1510s, 1464m, 1447m, 1397w, 1365m, 1302m, 1252s, 1202m, 1180s, 1157m, 1084m, 1035s, 979m, 899m, 880m, 829m, 791m, 758m, 727m, 700m, 641w, 585m, 523w.

4.2. Oligodeoxyonucleotide synthesis

Oligonucleotides were synthesized on the 1.0 µmol scale on a Expedite Nucleic Acid Synthesizer (8909) from *Applied Biosystems* using standard phosphoramidite chemistry. The phosphoramidites of the natural nucleosides were from *Glen Research*. The solvents and reagents used for the synthesis were prepared according to the manufacturer's protocols. 5-(Ethylthio)-1*H*-tetrazole (0.25 M in CH_3CN) was used as a coupling reagent and 3% dichloroacetic acid in CH_3CN was used for the detritylation step. After synthesis, the oligonucleotides were detached and deprotected in concd aq NH_3 (55 °C, 15 h) and filtered through *Titan HPLC-filters* (Teflon, 0.45 µm) from *Infochroma AG*. All oligodeoxynucleotides were purified by RP-HPLC on an *Äkta Basic 10/100* system from *Amersham Pharmacia Biotech* using a *15RPC ST 4.6/100* column from *Pharmacia Biotech* with a gradient of B: 0.1 M triethylammonium acetate in H_2O/CH_3CN 1:8, pH 7 in A: 0.1 M triethylammonium acetate in H_2O, pH 7. The flow rate was set to 1 ml/min. The detection wavelength was 260 and 280 nm, respectively. All oligodeoxynucleotides containing the novel modifications M and N were analyzed by ESIMS spectrometry (Table 2).

Table 2. Sequence and MS data for the oligodeoxynucleotides carrying the biphenyl-C-nucleotides **M** and **N**

	X,**Y**	*n*	*m*/*z* calcd.	*m*/*z* found	Yield ($O.D.^{260}$)
d(GATGAC$\mathbf{X}_n$GCTAG)	**M**	1	3727.4	3727.6	13.6
	M	3	4421.9	4422.2	17.8
	N	1	3757.5	3757.6	3.5
d(CTACTG$\mathbf{Y}_n$CGATC)	**M**	1	3638.4	3638.5	12.0
	M	3	4333.1	4333.2	11.4
	N	1	3668.6	3668.5	2.3

4.3. CD spectroscopy

Circular dichroism spectra were recorded on a *Jasco J-715* spectropolarimeter equipped with a *Jasco PFO-350S* temperature controller.

4.4. T_m Measurements

UV-melting curves were measured on a *Varian Cary 100 bio* UV–vis spectrophotometer. Absorbances of duplexes in a 1:1 stoichiometric ratio were monitored at 260 nm and the heating rate was set to 0.5 °C/min. Extinction coefficients for the novel C-nucleosides at 260 nm ($\varepsilon^{260\ nm}$) were determined to be 11,800 $M^{-1}\,cm^{-1}$ for **M** and 21,900 $M^{-1}\,cm^{-1}$ for **O**. A heating–cooling–heating cycle in the temperature range 10–90 °C was applied. The first derivative of the melting curves was obtained using *MicroCal Origin 5.0* software. Deviations of T_m values from repeated runs were found to be less than 0.5 °C. To avoid evaporation of the solution, dimethylpolysiloxane was layered over the sample solution in the UV cuvette.

4.5. Fluorescence measurements

Fluorescence spectra were measured on a *Varian Cary Eclipse* fluorescence spectrometer. The emission spectra were recorded at a scan speed of 600 nm per minute with a photomultiplier voltage of 700 V. The excitation and emission slits were set to 5 nm. The temperature-dependent measurements were recorded from 20 to 80 °C with a heating rate of 0.5 °C/min. After reaching the maximum temperature, the samples were cooled and another heating cycle was started (totally three cycles). To avoid evaporation of the solution, dimethylpolysiloxane was layered over the samples in the cell.

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