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Oligodeoxynucleotides containing α-L-*ribo* configured LNA-type *C*-aryl nucleotides †

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Synthesis of 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl derivatives containing phenyl and 1-pyrenyl aglycons, *i.e.*, novel α-L-*ribo* configured LNA-type *C*-aryl nucleosides, has been accomplished. Key synthetic steps included stereoselective Grignard reactions on tetrahydrofuran aldehyde **12**, configurational inversion of the resulting alcohol **13** into alcohol **15**, and concomitant Mitsonobu cyclization furnishing the desired bicyclic furanosyl skeleton with a locked conformation. The phosphoramidite derivatives **19a** and **19b** were used for automated synthesis of 9-mer DNA and α-L-LNA oligonucleotides containing the α-L-LNA-type *C*-aryl monomers $^{\alpha L}$**Ph**^{L} and $^{\alpha L}$**Py**^{L} containing a phenyl and pyrenyl aglycon, respectively. Thermal denaturation studies showed universal base pairing behavior for the pyrenyl monomer $^{\alpha L}$**Py**^{L} when incorporated into a DNA or an α-L-LNA oligonucleotide.

Introduction

An oligonucleotide containing a universal base monomer binds isoenergetically with the four oligonucleotide complements containing each of the natural nucleotides opposite to the universal base analogue.[1] Interesting universal bases include 2′-deoxy-β-D-ribofuranosyl derivatives of 3-nitropyrrole,[2] 5-nitroindole,[3] isocarbostyril[4] and 8-aza-7-deazaadenine.[5] Also pyrenyl derivatives[6–8] of 2′-deoxy-β-D-ribofuranose and LNA,[9]§ have been reported as universal bases.

Recently, we have reported universal base pairing behavior of the pyrenyl LNA monomer **Py**^{L} (Fig. 1) being locked in a C3′-*endo* RNA-like furanose conformation. The average duplex melting temperatures (T_m values) were decreased by ~10 °C relative to the native DNA-DNA duplex[7,8] which indicated **Py**^{L} as being more destabilizing than the corresponding pyrenyl DNA monomer for which decreased T_m values of ~5 °C were reported in a similar sequence context.[6] In order to study the relation between conformation and the effect of a pyrenyl unit in relation to universal hybridization, we decided to incorporate the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} containing a 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl moiety (Fig. 1).[10]¶ As CD and NMR spectroscopy studies of α-L-LNA:DNA duplexes have shown the overall duplex geometry to be of the B-type with preserved Watson–Crick base pairing, α-L-LNA can be most adequately described as a DNA mimic.[11] Therefore, the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} should furnish information on the effect of the pyrenyl aglycon when locked in a DNA-like conformation, and allow direct comparison with **Py**^{L} locked in an RNA-like conformation.[7,8] In addition, the corresponding phenyl analogue $^{\alpha L}$**Ph**^{L} (Fig. 1) was also synthesized.

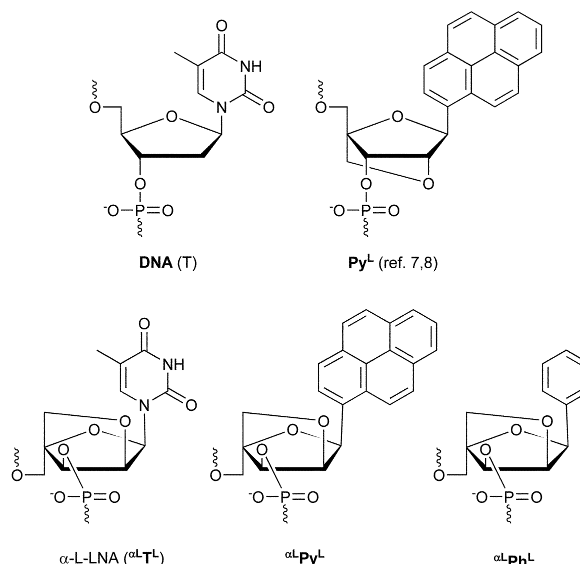

Fig. 1 Structures of nucleotide monomers: DNA (T), α-L-LNA ($^{\alpha L}$**T**^{L}),[10] LNA-type pyrenyl monomer (**Py**^{L})[7,8] and α-L-LNA-type pyrenyl ($^{\alpha L}$**Py**^{L}) and phenyl ($^{\alpha L}$**Ph**^{L}) monomers. The short notations shown are used in Table 1. For DNA and α-L-LNA, the thymine monomers are shown.

† Electronic supplementary information (ESI) available: Copies of the ^{13}C NMR spectra of compounds **1**–**4**, **5**–**11** (of both isomers), **12**,**13**–**18** (of phenyl and pyrenyl derivatives) and copies of the ^{31}P NMR spectra of compounds **19a** and **19b**. See http://www.rsc.org/suppdata/ob/b3/b310719a/

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§ We have defined LNA as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomer(s) (ref. 9). Analogously, α-L-LNA has been defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl nucleotide monomer(s) (ref. 10).

¶ Despite the apparent differences in the furanose conformations between an LNA monomer and an α-L-LNA monomer, also the furanose conformation of an α-L-LNA monomer is of the *N*-type (C3′-*endo*, ^{3}E) because of its L-configuration. For further information about the conformations of nucleotides, see *Eur. J. Biochem.* 1983, **131**, 9 (“Abbreviations and Symbols for the Description of Conformations of Polynucleotide Chains”; IUPAC-IUB Joint Commission on Biochemical Nomenclature).

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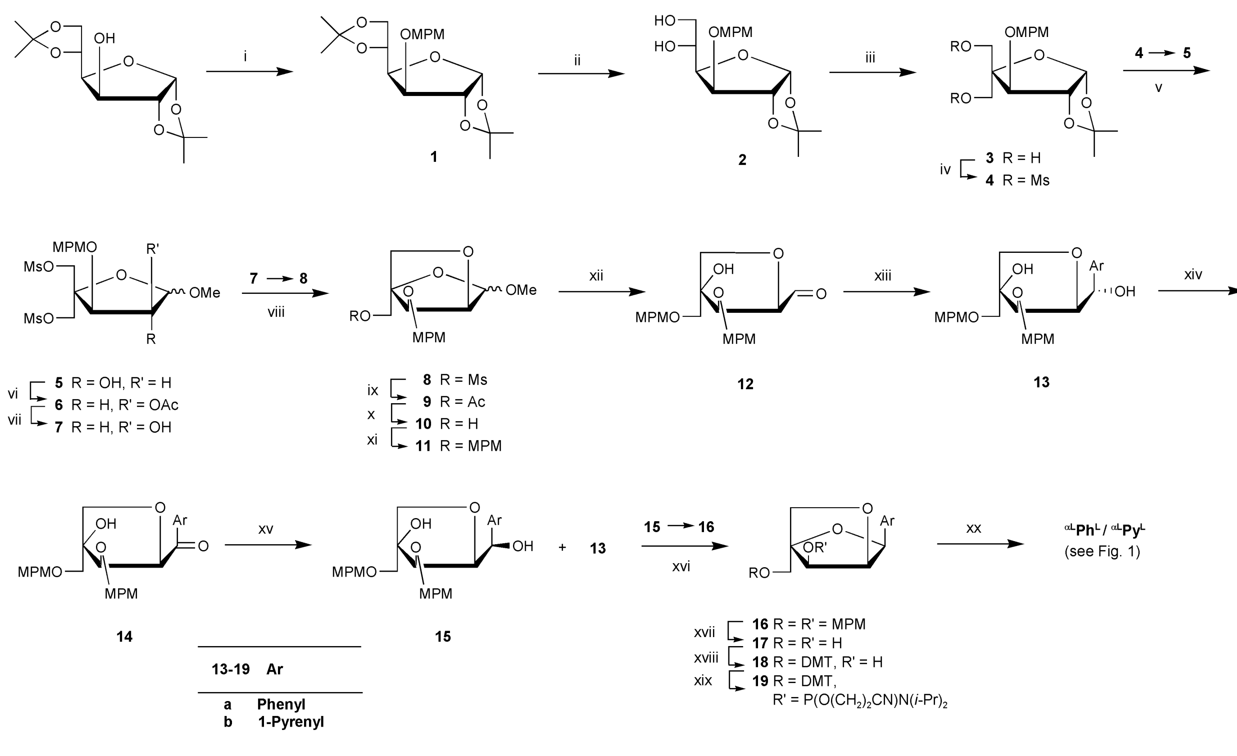


Scheme 1 *Reagents and conditions (and yields)*: (i) *p*-Methoxybenzyl chloride, NaH, THF, $(n\text{-Bu})_4N^+I^-$ (92%); (ii) 60% AcOH (82%); (iii) (a) $NaIO_4$, H_2O, THF, (b) HCHO, 2 M aq. NaOH, dioxane (87%); (iv) MsCl, pyridine (93%); (v) H_2O–HCl–CH_3OH (1 : 1.5 : 8.5, v/v/v) (major isomer **5a**: 57% + minor isomer **5b**: 26%); (vi) (a) Tf_2O, pyridine, (b) KOAc, 18-crown-6 ether, toluene (**6a**: 83%; **6b** 77%); (vii) sat. methanolic ammonia; (viii) NaH, DMF (**8a**: 72% (from **6a**); **8b**: 69% (from **6b**); (ix) KOAc, 18-crown-6 ether, dioxane (**9a**: 92%; **9b**: 78%); (x) sat. methanolic ammonia (**10a**: 96%; **10b**: 90%); (xi) *p*-methoxybenzyl chloride, NaH, THF, $(n\text{-Bu})_4N^+I^-$ (**11a**: 86%; **11b**: 81%); (xii) 70% AcOH (85% from **11a**; 80% from **11b**); (xiii) ArMgBr, THF (**13a**: 74%; **13b**: 71%); (xiv) Dess–Martin periodinane, CH_2Cl_2 (**14a**: 76%); PDC, 3Å molecular sieves, CH_2Cl_2 (**14b**); (xv) $NaBH_4$, THF, H_2O (**15a**: 22%; **15b**:16% (from **13b**)); (xvi) TMAD, Bu_3P, benzene (**16a**: 97%; **16b**: 88%); (xvii) DDQ, CH_2Cl_2, H_2O (**17a**: 63%; **17b**: 52%); (xviii) DMTCl, pyridine (**18a**: 88%; **18b**: 54%); (xix) $NC(CH_2)_2OP(Cl)N(i\text{-Pr})_2$, $EtN(i\text{-Pr})_2$, CH_2Cl_2 (**19a**: 57%; **19b**: 42%); (xx) DNA synthesizer.

Results and discussion

Synthesis of phosphoramidite building blocks 19a and 19b

As an appropriate synthetic route, the protocol shown in Scheme 1, based on earlier syntheses published for *C*-aryl nucleosides of LNA,[7,8,12–14] was used (yields are shown in the caption of Scheme 1). 1,2:5,6-Di-*O*-isopropylidene-α-D-glucose was *p*-methoxybenzylated to give furanoside **1** followed by selective cleavage of the 5,6-*O*-isopropylidene group with 60% acetic acid at room temperature furnishing diol **2**. Periodate cleavage of **2** and subsequent *in situ* aldol condensation and Cannizzaro reaction with formaldehyde under alkaline conditions afforded furanoside **3**. Di-O-mesylation followed by methanolysis afforded a (1 : 2.2) anomeric mixture of methyl furanoside **5** which subsequently was separated by column chromatography. The single anomers were reacted individually in the following steps. Epimerization at C2 to give 2-*O*-acetyl derivatives **6** was achieved by activation with trifluoromethanesulfonic anhydride followed by nucleophilic substitution by reaction with potassium acetate in the presence of 18-crown-6 ether. Deacetylation with saturated methanolic ammonia yielded mainly furanoside **7** along with traces of the bicyclic compound **8**. Analytical samples were obtained after column chromatographic purification, and the crude mixture was directly cyclized in the presence of sodium hydride in DMF to give the 2,5-dioxabicyclo[2.2.1]heptane derivative **8**. Substitution of the remaining mesyloxy group of **8** with an acetate group, followed by deacetylation and then *p*-methoxybenzylation yielded the furanoside **11** (*via* **9** and **10**). Due to ring strain, acetal hydrolysis cleanly afforded the monocyclic aldehyde **12** which served as a general electrophile for Grignard additions as reported earlier for derivatives in the enantiomeric series.[7,8,12–14] Reaction of this aldehyde with phenyl or pyrenyl Grignard reagents yielded diastereoselectively a single product assigned as the *S*-epimer based upon the fact that similar Grignard reactions on the enantiomeric aldehyde have been reported to exclusively furnish the *R*-epimeric products.[7,8] This stereochemical assignment was later confirmed (see below) by an NOE experiment on the bicyclic nucleoside **16**. For the synthesis of the desired 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl nucleosides another epimerization was required, this time at C1. *In situ* oxidation of the phenyl analogue **13a** with Dess–Martin periodinane (to give ketone **14**) followed by reduction with sodium borohydride afforded a 1 : 2.4 separable mixture of diastereomers. Although the epimerized product **15a** was isolated in only 22% yield this route was preferred over the alternative, but more complicated, process involving selective 4-O-protection, 1-O-activation, inversion and deprotection. Moreover, the other epimer **13a** was recovered. Surprisingly, oxidation of the pyrenyl analogue **13b** with the Dess–Martin reagent did not afford the required oxidation product **14b**, probably due to the formation of acetic acid during the course of the reaction as it was later observed that **14b** is unstable even on silica gel. Instead, the oxidation was carried out under neutral conditions with pyridinium dichromate as oxidant and the crude product obtained was directly reduced without purification to afford a 1 : 2 mixture of epimers **15b** and **13b** which were separated by column chromatography. The diols **15a** and **15b** were efficiently cyclized under Mitsunobu conditions to afford the bicyclic α-L-configured *C*-aryl nucleosides **16a** and **16b**, respectively. The assigned configuration of compound **16a** was verified by an NOE difference experiment. Thus, selective irradiation of one of the H5″ protons (appearing at δ 4.21) gave a 13.2% enhancement of the signal of the pyrenyl moiety and *vice versa* (5.8%). In addition, very significant and mutual NOE effects were observed between H1′ and H3′ (11.3/8.7%) which besides verification of configuration strongly suggest that the

Table 1 Thermal denaturation experiments (T_m values shown) for **ON1**–**ON6** towards DNA complements with each of the four natural bases in the central position[a]

		Y			
DNA Target	3′-d(CACT**Y**TACG)	A	C	G	T
ON1	5′-d(GTGATATGC)	27[b]/36[c]			
ON2	5′-d(GTGA**Py**^{L}ATGC)[7,8]	18[b]	17[b]	18[b]	19[b]
ON3	5′-d(GTGA$^{\alpha L}$**Ph**^{L}ATGC)	<5[b]/12[c]	<5[b,c]	<5[b,c]	<5[b]/12[c]
ON4	5′-d(GTGA$^{\alpha L}$**Py**^{L}ATGC)	21[b]/30[c]	22[b]/31[c]	27[b]/34[c]	23[b]/33[c]
ON5	5′-d(G$^{\alpha L}$**T**^{L}GA$^{\alpha L}$**Ph**^{L}A$^{\alpha L}$**T**^{L}GC)	<5[b]/14[c]	<5[b,c]	<5[b,c]	<5[b]/13[c]
ON6	5′-d(G$^{\alpha L}$**T**^{L}GA$^{\alpha L}$**Py**^{L}A$^{\alpha L}$**T**^{L}GC)	24[b]/33[c]	23[b]/33[c]	31[b]/36[c]	26[b]/34[c]

[a] Melting temperatures (T_m values/°C) measured as the maximum of the first derivative of the melting curve (A_{260} *vs.* temperature) recorded in medium salt buffer. [b] (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) or in high salt buffer. [c] (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.0 μM concentrations of the two strands; A = adenin-9-yl monomer, C = cytosin-1-yl monomer, G = guanin-9-yl monomer, T = thymin-1-yl; see Fig. 1 for structures of $^{\alpha L}$**T**^{L},$^{\alpha L}$ **Ph**^{L}, **Py**^{L} and $^{\alpha L}$**Py**^{L}.

bicyclic nucleoside adopts the expected *N*-type (C3′-*endo*) furanose conformation.¶ Similarly for the bicyclic phenyl derivative **16b** mutual NOE enhancements were observed between H1′ and H3′ (10.7/9.1%) and between protons of the phenyl moiety and the H5″ protons (1.4/2.7%). Oxidative removal of the *p*-methoxybenzyl protection groups was achieved with DDQ yielding derivatives **17a** and **17b**. To prepare for automated synthesis of oligonucleotides containing the phenyl ($^{\alpha L}$**Ph**^{L}) and pyrenyl ($^{\alpha L}$**Py**^{L}) α-L-LNA monomers, the primary-hydroxy group of nucleoside diols **17a** and **17b** was selectively protected by reaction with 4,4′-dimethoxytrityl chloride (to give compounds **18a** and **18b**, respectively) followed by phosphitylation under standard conditions to furnish the desired phosphoramidite building blocks **19a** and **19b** in satisfactory yields (Scheme 1).

Synthesis of oligonucleotides containing *C*-phenyl monomer $^{\alpha L}$Ph^{L} and *C*-pyrenyl monomer $^{\alpha L}$Py^{L}

All oligonucleotides **ON3**–**ON6** (Table 1) were prepared in 0.2 μmol scale using the phosphoramidite approach (see the Experimental section for details). The stepwise coupling efficiencies of phosphoramidites **19a** and **19b** (10 min coupling time) and of unmodified deoxynucleoside phosphoramidites (2 min coupling time) were >99% using 1*H*-tetrazole as activator (pyridine hydrochloride was used as activator when coupling **19b**). After standard deprotection and cleavage from the solid support using 32% aqueous ammonia (12 h, 55 °C), the oligomers were purified by precipitation from ethanol. The composition of oligomers **ON3**–**ON6** was verified by MALDI-MS analysis and their purity (>80%) by capillary gel electrophoresis.

Thermal denaturation studies and fluorescence measurements

The hybridization of the novel modified oligonucleotides **ON3**–**ON6** (Table 1) towards four 9-mer DNA targets with the central base being each of four natural bases was studied by thermal denaturation experiments at medium and high salt buffer conditions (see the Experimental section for details). The obtained melting temperatures (T_m values) were compared with those obtained for the DNA reference **ON1** (towards the fully matched target) and with those reported for the pyrenyl LNA monomer **Py**^{L} showing universal hybridization.[7,8] Introduction of the phenyl α-L-LNA monomer $^{\alpha L}$**Ph**^{L} (**ON3**) reduces the thermal stability of the resulting duplexes very significantly even at high salt conditions and even when combined with two thymine α-L-LNA monomers (monomers $^{\alpha L}$**T**^{L}, **ON5**) known to be affinity enhancing in this sequence context.[15] Interestingly, incorporation of one pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} (**ON4**) induces T_m values in both medium and high salt conditions being only moderately reduced relative to, or similar to, the DNA reference **ON1**. In this case, the introduction of two thymine α-L-LNA monomers (**ON6**) has a weak positive effect on the T_m values. It is apparent from the studies on **ON4** and **ON6** that the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} displays a subtle preference for "pairing" with a guanine monomer, and that this effect is most pronounced under medium salt conditions.

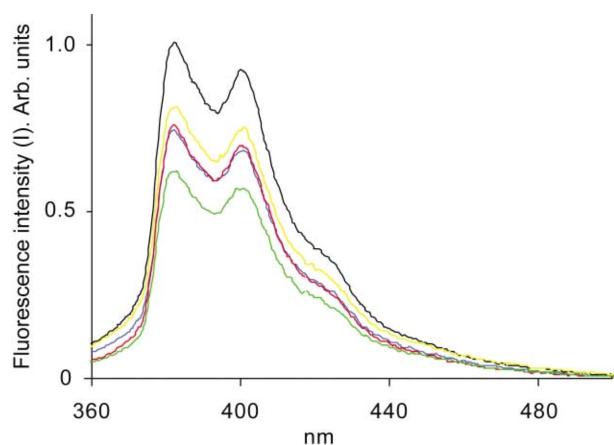
Comparison of the pyrenyl LNA monomer **Py**^{L} (**ON2**)[7,8] and the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} (**ON4**) reveals several interesting differences. Under medium salt conditions, the affinity was determined[7,8] to be significantly lower for **Py**^{L} but the hybridization with the four DNA target strands more universal. However, the performance of the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} as a universal base is improved by changing into high salt conditions (T_m values = 30–34 °C (**ON4**) and 33–36 °C (**ON6**).

Fluorescence spectra of single stranded **ON4** and of duplexes formed between **ON4** and the four 9-mer DNA targets were measured (Fig. 2). In general, the spectra show the characteristics of pyrene monomer emission, *i.e.*, two bands (band I and III) with emission maxima at 383 and 401 nm, respectively. The fluorescence intensity of single stranded **ON4** is about 45% of that of the monomer **17b**, and stronger than the intensities obtained for the four duplexes (Fig. 2). These facts support intercalation of the pyrene aglycon of $^{\alpha L}$**Py**^{L}, especially in the case of the duplexes resulting in more efficient fluorescence quenching by the nucleobases than for the more flexible single stranded **ON4**. It is noticeable that the minimum fluorescence intensity is obtained for the thermally most stable duplex which underlines the importance of intercalation and/or reduced flexibility for fluorescence quenching.

Anticipating intercalation, at least in part, of the pyrenyl moiety of $^{\alpha L}$**Py**^{L} into the nucleobase stack, the results obtained herein show pyrenyl intercalation and nucleobase interaction to be more efficient in the DNA-like context of **ON4** than in the RNA-like context of the corresponding LNA (**Py**^{L}).[7,8] A similar trend has been reported for other pyrene-containing monomers.[16–19] However, comparison of relative melting temperatures obtained with the pyrenyl α-L-LNA monomer $^{\alpha L}$**Py**^{L} and the corresponding pyrenyl DNA monomer[6] reveals no affinity enhancing effect of conformational restriction. It is plausible that the limited flexibility imposed by the locked 2,5-dioxabicyclo[2.2.1]heptane skeleton impedes optimal intercalation of the pyrenyl moiety.

Conclusion

Synthesis of phenyl and pyrenyl derivatives of α-L-LNA-type *C*-aryl nucleosides has been accomplished as has their efficient incorporation into 9-mer DNA and α-L-LNA strands. For the pyrenyl derivative, universal hybridization towards DNA targets was obtained under high salt conditions ([Na^+] = 710 mM). Fluorescence measurements indicated intercalation of the pyrenyl moiety into the nucleobase stack of the duplexes.



Color code	Sample	I_M	
		Band I (I_I)	Band III (I_{III})
♦	GTG $A^{\alpha L}$**Py**^{L}A TGC	1	0.92
♦	GTG $A^{\alpha L}$**Py**^{L}A TGC: CAC TAT GCA	0.81	0.75
♦	GTG $A^{\alpha L}$**Py**^{L}A TGC: CAC TCT GCA	0.76	0.70
♦	GTG $A^{\alpha L}$**Py**^{L}A TGC: CAC TGT GCA	0.62	0.57
♦	GTG $A^{\alpha L}$**Py**^{L}A TGC: CAC TTT GCA	0.74	0.68

Fig. 2 Steady state fluorescence spectra of single stranded **ON4** and of the four duplexes shown. The spectra were recorded at 19 °C ± 1 °C in buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5×10^{-7} M concentrations of each strand. Fluorescence intensities are arbitrary units normalized relative to the fluorescence intensity of band I of **ON4** (assigned the value "1").

Experimental

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with fluorescence indicator (SiO_2-60, F-254) which were visualized under UV light and by spraying with 5% conc. sulfuric acid in absolute ethanol (v/v) followed by heating. Silica gel 60 (particle size 0.040–0.063 mm, Merck) was used for column chromatography. Light petroleum of the distillation range 60–80 °C was used. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure and dried 12 h under high vacuum to give the product unless otherwise specified. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H NMR and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR. Assignments of NMR spectra, when given, are based on 2D NMR experiments (the assignments of methylene protons/carbons may be interchanged). Coupling constants (J values) are given in Hertz. Bicyclic compounds are named according to the Von Bayer nomenclature, whereas the atom numbering follows the standard carbohydrate/nucleoside nomenclature. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a Kratos MS50TC spectrometer and MALDI-HRMS were recorded in positive ion mode on a IonSpec Fourier transform mass spectrometer. The composition of the oligonucleotides was verified by MALDI-MS on a Micromass Tof Spec E mass spectrometer using a matrix of diammonium citrate and 2,6-dihydroxyacetophenone.

3-*O*-(*p*-Methoxybenzyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (1). Sodium hydride (4.5 g, 60% suspension in mineral oil, 112.5 mmol) was added to anhydrous THF (30 cm^3) under an inert atmosphere and the suspension was cooled to 0 °C. A solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (19.5 g, 74.9 mmol) in anhydrous THF (20 cm^3) was added dropwise over 30 min. The temperature of the mixture was allowed to raise to rt and stirring was continued for 15 min. *p*-Methoxybenzyl chloride (14.5 g, 92.6 mmol) was added dropwise followed by tetrabutylammonium iodide (8.6 g, 23.3 mmol) and the contents were stirred for 36 h at rt. The mixture was cooled to 0 °C and ice-cold water (100 cm^3) was carefully added. Extraction was performed with EtOAc (2 × 100 cm^3) and the combined organic phase was washed with brine (60 cm^3), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [8–10% (v/v) EtOAc in light petroleum] to give furanoside **1** as a colourless oil (26.2 g, 92%). R_f 0.33 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.27 (2H, d, *J* 8.7, Ar-H), 6.87 (2H, d, *J* 8.6, Ar-H), 5.88 (1H, d, *J* 3.8, H-1), 4.63–4.54 (3H, m, H-2 and CH_2 (MPM)), 4.33 (1H, m, H-4), 4.16–4.08 (2H, m, H-3 and H-6a), 4.02–3.97 (2H, m, H-5 and H-6b), 3.80 (3H, s, OCH_3), 1.49, 1.43, 1.38, 1.31 (3H each, 4s, CH_3 (isopropylidene)); δ_C ($CDCl_3$) 159.5, 129.8, 129.4 and 113.9 (ArH), 111.8 and 109.0 (O*C*(CH_3)$_2$O), 105.4 (C-1), 82.8 (C-2), 81.4 (C-3 and C-5), 72.7 (C-4), 72.2 (CH_2 (MPM)), 67.5 (C-6), 55.4 (OCH_3), 26.9, 26.8, 26.3 and 25.6 (CH_3 (isopropylidene)), MALDI-HRMS: *m*/*z* 403.1714 ([M + Na]$^+$, $C_{20}H_{28}O_7Na^+$ calc. 403.1727).

1,2-Di-*O*-isopropylidene-3-*O*-(*p*-methoxybenzyl)-α-D-glucofuranose (2). 60% Acetic acid (300 cm^3) was added to furanoside **1** (26.0 g, 68.3 mmol) and the reaction mixture was stirred at rt for 24 h. The reaction mixture was washed with light petroleum (2 × 100 cm^3) and subsequently concentrated to dryness under reduced pressure and co-evaporated successively with absolute ethanol (2 × 50 cm^3) and toluene (2 × 50 cm^3). The residue was dissolved in CH_2Cl_2 (200 cm^3) and washed with sat. aq. $NaHCO_3$ (2 × 100 cm^3). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure to give a residue which was purified by column chromatography [40% (v/v) EtOAc in light petroleum] to give furanoside **2** as a colourless oil (19.1 g, 82%). R_f 0.28 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.27 (2H, d, *J* 8.7, Ar-H), 6.89 (2H, d, *J* 8.3, Ar-H), 5.92 (1H, d, *J* 3.6, H-1), 4.66 (1H, d, *J* 11.6, CH_2 (MPM)), 4.60 (1H, d, *J* 3.9, H-2), 4.47 (1H, d, *J* 11.6, CH_2 (MPM)), 4.13–4.07 (2H, m, H-3 and H-4), 4.00 (1H, m, H-5), 3.79 (3H, s, OCH_3), 3.77 (1H, m, H-6a), 3.67 (1H, m, H-6b), 2.68 (1H, d, *J* 5.7, 5-OH), 2.44 (1H, br s, 6-OH), 1.48 and 1.31 (3H each, 2s, CH_3 (isopropylidene)); δ_C ($CDCl_3$) 159.7, 129.7, 129.3 and 114.2 (Ar), 111.9, (O*C*(CH_3)$_2$O), 105.2 (C-1), 82.2 (C-2), 81.6 and 80.0 (C-3 and C-4), 71.8 (CH_2 (MPM)), 69.4 (C-5), 64.4 (C-6), 55.4 (OCH_3), 26.8 and 26.3 (CH_3 (isopropylidene)); MALDI-HRMS: *m*/*z* 363.1400 ([M + Na]$^+$, $C_{17}H_{24}O_7Na^+$ calc. 363.1414).

1,2-*O*-Isopropylidene-4-*C*-hydroxymethyl-3-*O*-(*p*-methoxybenzyl)-β-L-*threo*-pentofuranose (3). Sodium periodate (38 g, 177.7 mmol) was added slowly to a stirred solution of furanoside **2** (50.0 g, 146.9 mmol) in a (1 : 1) mixture of THF-H_2O (360 cm^3) at 0 °C. The reaction mixture was stirred 12 h at rt. The white precipitate formed was filtered off and washed with EtOAc (3 × 50 cm^3). The aqueous phase was separated and extracted with EtOAc (2 × 60 cm^3), and the combined organic phase was washed with brine (2 × 50 cm^3) and evaporated to dryness under reduced pressure. The viscous oil obtained was dissolved in dioxane (130 cm^3) and formaldehyde (37% solution in water, 34.4 cm^3, 391.4 mmol) and sodium hydoxide (2 M solution, 150 cm^3) were added. The reaction mixture was stirred for 24 h at rt whereupon extraction was performed with CH_2Cl_2

(2×200 cm^3). The combined organic phase was washed with brine (150 cm^3), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [50% (v/v) EtOAc in light petroleum] to give furanoside **3** as a white solid material (43.3 g, 87%).R_f 0.28 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.25 (2H, d, *J* 8.6, Ar-H), 6.89 (2H, d, *J* 8.5, Ar-H), 6.00 (1H, d, *J* 4.4, H-1), 4.74 (dd, 1H, *J* 1.5 and 4.5, H-2), 4.69 (1H, d, *J* 11.3, CH_2 (MPM)), 4.47 (1H, d, *J* 11.3, CH_2 (MPM)), 4.09 (1H, d, *J* 1.9, H-3), 3.81 (3H, s, OCH_3), 3.72–3.60 (4H, m, H-5 and H-5′), 2.44–2.41 (2H, m, 2 × OH), 1.54 and 1.35 (3H each, 2 s, CH_3 (isopropylidene)); δ_C ($CDCl_3$) 159.7, 129.6, 129.0 and 114.1 (Ar), 113.2 ($OC(CH_3)_2O$), 105.0 (C-1), 89.9 (C-4), 86.0 (C-2), 84.6 (C-3), 72.4 (CH_2 (MPM)), 63.9 and 63.5 (C-5 and C-5′), 55.4 (OCH_3), 27.4 and 26.9 (CH_3 (isopropylidene)); MALDI-HRMS: *m*/*z* 363.1402 ([M + Na]$^+$, $C_{17}H_{24}O_7Na^+$ calc. 363.1414).

1,2-*O*-Isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-β-L-*threo*-pentofuranose (4). Methanesulfonyl chloride (6.39 g, 55.8 mmol) was added dropwise to a stirred solution of furanoside **3** (7.60 g, 22.3 mmol) in a mixture of anhydrous pyridine (10 cm^3) and CH_2Cl_2 (10 cm^3). The reaction mixture was stirred 12 h at rt. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (50 cm^3) and washed with sat. aq. $NaHCO_3$ (2×25 cm^3). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [50% (v/v) EtOAc in light petroleum] affording furanose **4** which was crystallized from EtOH as a white solid material (10.3 g, 93%). R_f 0.45 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26.(2H, d, *J* 8.5, Ar-H), 6.90 (2H, d, *J* 8.8, Ar-H), 5.97 (1H, d, *J* 3.9, H-1), 4.69 (1H, d, *J* 3.9, H-2), 4.64 (1H, d, *J* 11.3, CH_2 (MPM)), 4.48 (1H, d, *J* 11.2, CH_2 (MPM)), 4.39–4.30 (4H, m, H-5 and H-5′), 4.07 (1H, s, H-3), 3.81 (3H, s, OCH_3), 3.03 (3H, s, SO_2CH_3), 3.00 (3H, s, SO_2CH_3), 1.57 and 1.32 (3H each, 2s, (CH_3(isopropylidene)); δ_C ($CDCl_3$) 159.8, 129.9, 128.4 and 114.1 (Ar), 113.3 ($OC(CH_3)_2O$), 105.9 (C-1), 86.1 (C-4), 84.3 (C-2), 82.5 (C-3), 72.7 (CH_2 (MPM)), 67.5 and 67.3 (C-5 and C-5′), 55.4 (OCH_3), 37.7 and 37.5 (2 × SO_2CH_3), 26.6 and 26.0 (CH_3 (isopropylidene)); MALDI-HRMS: *m*/*z* 519.0965 ([M + Na]$^+$, $C_{19}H_{28}O_{11}S_2Na^+$ calc. 519.0965).

Methyl 5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-α,β-L-*threo*-pentofuranoside (5). A suspension of furanoside **4** (10.5 g, 21.2 mmol) in a mixture of concentrated aqueous HCl and MeOH (380 cm^3, 3 : 17, v/v) was stirred at rt for 36 h. The mixture was carefully neutralized by addition of $NaHCO_3$ (s), whereupon the mixture was evaporated to dryness under reduced pressure. H_2O (100 cm^3) was added to the residue obtained and extraction was performed with EtOAc (3×100 cm^3). The combined organic phase was washed with brine (100 cm^3), dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was coevaporated with toluene (2×25 cm^3) and purified by column chromatography [45–50% (v/v) EtOAc in light petroleum] to give first the minor isomer **5b** (2.6 g, 26%) and then [50–60% (v/v) EtOAc in light petroleum] the major isomer **5a** (5.7 g, 57%) both as a clear oil. R_f 0.52, 0.43 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$, major isomer **5a**) 7.27 (2H, d, *J* 8.8, Ar-H), 6.89 (2H, d, *J* 8.8, Ar-H), 4.89 (1H, d, *J* 4.8, H-1), 4.74 (1H, d, *J* 11.5, CH_2 (MPM)), 4.59 (1H, d, *J* 11.5, CH_2 (MPM)), 4.35 (1H, d, *J* 11.0, H-5a), 4.31 (1H, m, H-2), 4.27 (1H, d,*J* 10.9, H-5b), 4.24 (1H, d, *J* 10.2, H-5′a), 4.06 (1H, d, *J* 10.1, H-5′b), 3.98 (1H, d, *J* 7.0, H-3), 3.80 (3H, s, OCH_3 (MPM)), 3.46 (3H, s, OCH_3), 3.02 and 2.96 (3H each, 2s, SO_2CH_3), 2.54 (1H, d, *J* 9.7, 2-OH); δ_C ($CDCl_3$, major isomer) 159.6, 129.8, 129.2 and 114.0 (Ar), 101.8 (C-1), 84.6 (C-3), 80.9 (C-4), 77.5 (C-2), 72.6 (CH_2 (MPM)), 69.5 and 68.7 (C-5 and C-5′), 56.1 (OCH_3), 55.3 (OCH_3 (MPM)), 37.6 and 37.5 (2 × SO_2CH_3); MALDI-HRMS: *m*/*z* 493.0815 ([M + Na]$^+$, $C_{17}H_{26}O_{11}S_2Na^+$ calc. 493.0809); δ_H ($CDCl_3$, minor isomer **5b**) 7.27 (2H, d, *J* 8.5, Ar-H), 6.90 (2H, d, *J* 8.6, Ar-H), 4.87 (1H, s, H-1), 4.63 (1H, d, *J* 11.7, H-5a), 4.51 (1H, d, *J* 11.7, H-5b), 4.44 (1H, d, *J* 10.7, H-5′a), 4.35 (1H, d,*J* 10.6, H-5′b), 4.26 (3H, m, H-2 and CH_2 (MPM)), 3.97 (1H, d, *J* 1.9, H-3), 3.81 (3H, s, OCH_3 (MPM)), 3.41 (3H, s, OCH_3), 3.01 and 3.00 (3H each, 2s, 2 × SO_2CH_3), 2.47 (1H, d, *J* 4.3, 2-OH); δ_C ($CDCl_3$, minor isomer) 159.7, 129.9, 129.1, 114.1, 110.2, 85.3, 84.3, 79.9, 72.9, 69.1, 68.9, 55.9, 55.4, 37.6 and 37.3; MALDI-HRMS: *m*/*z* 493.0817 ([M + Na]$^+$, $C_{17}H_{26}O_{11}S_2Na^+$ calc. 493.0809).

Methyl 2-*O*-acetyl-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-α,β-L-*erythro*-pentofuranoside (6). To a stirred solution of furanoside **5a** (18.1 g, 38.5 mmol) in pyridine (38 cm^3) and CH_2Cl_2 (176 cm^3) was dropwise added triflic anhydride (13.0 g, 46.1 mmol) at −30 °C over 30 min. The resulting mixture was stirred for 12 h at rt and then washed with sat. aq. $NaHCO_3$ (2×100 cm^3), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2×25 cm^3) and then dissolved in a mixture of CH_2Cl_2 (53 cm^3) and toluene (400 cm^3). 18-crown-6 ether (32 g, 121.0 mmol) and KOAc (12.8 g, 130.4 mmol) were added to the stirred solution. After 12 h at 50 °C, the reaction mixture was washed with sat. aq. $NaHCO_3$ (2×100 cm^3), dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography [30–35% (v/v) EtOAc in light petroleum] to give furanoside **6a** as a white solid material (16.4 g, 83%). R_f 0.69 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.23 (2H, d, *J* 8.3, Ar-H), 6.88 (2H, d, *J* 8.7, Ar-H), 5.27 (1H, d, *J* 4.8, H-2), 4.90 (1H, s, H-1), 4.52 (1H, d, *J* 10.8, CH_2 (MPM)), 4.46–4.31 (5H, m, H-3, H-5, H-5′a and CH_2 (MPM)), 4.09 (1H, d, *J* 9.9, H-5′b), 3.80 (3H, s, OCH_3 (MPM)), 3.36 (3H, s, OCH_3), 3.03 and 3.01 (3H each, 2s, 2 × SO_2CH_3), 2.13 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 169.6 ($COCH_3$), 159.8, 130.0, 128.8 and 114.0 (Ar), 105.9 (C-1), 81.3 (C-4), 79.9 (C-3), 74.0 (C-2), 73.5 (CH_2 (MPM)), 69.3 and 69.1 (C-5 and C-5′), 55.6 (OCH_3), 55.4 (OCH_3 (MPM)), 37.6 and 37.5 (2 × SO_2CH_3), 20.8 ($COCH_3$); MALDI-HRMS: *m*/*z* 535.0913 ([M + Na]$^+$, $C_{19}H_{28}O_{12}S_2Na^+$ calc. 535.0914); Similarly the minor isomer **5b** (9.0 g, 19.2 mmol) was activated with triflic anhydride (7.0 g, 24.8 mmol) in the presence of pyridine (20 cm^3), followed by epimerization with KOAc (6.42 g, 65.4 mmol) in the presence of 18-crown-6 ether (17.6 g, 66.6 mmol). The residue obtained after work up procedure was purified by column chromatography [40–50% (v/v), EtOAc in light petroleum] to give furanoside **6b** as a white solid material (7.55 g, 77%). R_f 0.58 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.27 (2H, d, *J* 8.3, Ar-H), 6.89 (2H, d, *J* 8.0, Ar-H), 5.12 (1H, d, *J* 4.4, H-1), 4.95 (1H, dd, *J* 5.0 and 6.3, H-2), 4.65 (1H, d, *J* 11.4, H-5a), 4.50 (1H, d, *J* 11.1, H-5′a), 4.43 (1H, d, *J* 11.7, H-5b), 4.24 (1H, d, *J* 6.8, H-3), 4.19 (1H, *J* 10.8, H-5′b), 4.12 (2H, s, CH_2 (MPM)), 3.81 (3H, s, OCH_3 (MPM)), 3.46 (3H, s, OCH_3), 3.00 and 2.99 (3H each, 2s, 2 × SO_2CH_3), 2.16 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 170.2 ($COCH_3$), 159.9, 130.3, 128.8 and 114.1 (Ar), 102.7 (C-1), 82.7 (C-4), 76.5 (C-3), 74.6 (CH_2 (MPM)), 71.7 (C-2), 69.3 and 68.8 (C-5 and C-5′), 56.5 (OCH_3), 55.4 (OCH_3 (MPM)), 37.6 and 37.3 (SO_2CH_3), 20.8 ($COCH_3$); MALDI-HRMS: *m*/*z* 535.0927 ([M + Na]$^+$, $C_{19}H_{28}O_{12}S_2Na^+$ calc. 535.0914).

Methyl 5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-α,β-L-*erythro*-pentofuranoside (7). A solution of furanoside **6a** (isomer obtained from the major anomer of **5**, 22.0 g, 42.9 mmol) in sat. methanolic ammonia (250 cm^3) was stirred for 24 h at rt (complete conversion of starting material into two products according to analytical TLC). The reaction mixture was evaporated to dryness under

reduced pressure and the residue was coevoporated with toluene (2 × 50 cm^3). An analytical sample was purified by column chromatography (30% (v/v) EtOAc in light petroleum) to give as the major product furanoside **7a**. R_f 0.53 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.28 (2H, d, *J* 8.3, Ar-H), 6.91 (2H, d, *J* 8.6, Ar-H), 4.87 (1H, s, H-1), 4.62 (1H, d, *J* 11.4, CH_2 (MPM)), 4.53 (1H, d, *J* 11.2, CH_2 (MPM)), 4.43 (1H, d, *J* 11.1, H-5a), 4.38 (1H, d, *J* 11.3, H-5b), 4.31 (1H, d, *J* 9.6, H-5′a), 4.25 (1H, d, *J* 4.9, H-3), 4.06 (1H, d, *J* 9.8, H-5′b), 3.98 (1H, dd, *J* 2.5 and 4.7, H-2), 3.81 (3H, s, OCH_3 (MPM)), 3.33 (3H, s, OCH_3), 3.06 (3H, s, SO_2CH_3), 3.03 (3H, s, SO_2CH_3), 2.55 (1H, d, *J* 2.5, 2-OH); δ_C ($CDCl_3$) 160.0, 130.1, 128.5 and 114.3 (Ar), 107.8 (C-1), 81.7 (C-4), 81.2 (C-3), 73.9 (C-2), 73.6 (CH_2 (MPM)), 69.7 and 69.5 (C-5 and C-5′), 55.5 (OCH_3), 55.4 (OCH_3 (MPM)), 37.5 (SO_2CH_3), 37.4 (SO_2CH_3); MALDI-HRMS: *m*/*z* 493.0811 ([M + Na]$^+$, $C_{17}H_{26}O_{11}S_2Na^+$ calc. 493.0808). The minor product was tentatively assigned as **8a** presumably formed by deacetylation followed by base induced intramolecular cyclization. The crude mixture of **7a** and **8a** was therefore used in the next step without purification. Similarly, deacetylation of the epimer **6b** (5.05 g, 9.85 mmol) using sat. methanolic ammonia (60 cm^3) yielded two products. An analytical sample was purified by column chromatography (35% (v/v) EtOAc in light petroleum) to give the major product **7b** as a white solid material. R_f 0.46 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.28 (2H, d, *J* 8.5, Ar-H), 6.91 (2H, d, *J* 8.5, Ar-H), 4.88 (1H, d, *J* 4.7, H-1), 4.70 (1H, d, *J* 11.3, CH_2 (MPM)), 4.52 (1H, d, *J* 11.4, CH_2 (MPM)), 4.44 (1H, d, *J* 10.8, H-5a), 4.20 (1H, d, *J* 11.0, H-5b), 4.18 (1H, m, H-2), 4.14 (1H, d, *J* 10.5, H-5′a), 4.08 (1H, d, *J* 10.6, H-5′b), 4.00 (1H, d, *J* 7.0, H-3), 3.81 (3H, s, OCH_3 (MPM)), 3.46 (3H, s, OCH_3), 3.01 (3H, s, SO_2CH_3), 3.00 (3H, s, SO_2CH_3), 2.88 (1H, d, *J* 11.2, 2-OH); δ_C ($CDCl_3$) 159.8, 130.2, 128.9 and 114.2 (Ar), 103.6 (C-1), 83.2 (C-4), 77.7 (C-3), 75.0 (C-2), 71.9 (CH_2 (MPM)), 69.2 and 69.1 (C-5 and C-5′), 56.3 (OCH_3), 55.4 (OCH_3 (MPM)), 37.6 (SO_2CH_3), 37.4 (SO_2CH_3); MALDI-HRMS: *m*/*z* 493.0819 ([M + Na]$^+$, $C_{17}H_{26}O_{11}S_2Na^+$ calc. 493.0809).

(1*S*,3*RS*,4*S*,7*R*)-1-Methanesulfonyloxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (8). The crude mixture (~23.0 g) obtained from methanolic ammonia treatment of **6a** was dissolved in anhydrous DMF (50 cm^3) and the resulting mixture was cooled to 0 °C. Sodium hydride (4.0 g, 60% suspension in mineral oil (w/w), 100 mmol) was added slowly during 10 min and the mixture was stirred for 12 h at rt whereupon ice-cold H_2O (200 cm^3) was carefully added. The resulting mixture was extracted using EtOAc (3 × 100 cm^3) and the combined organic phase was washed successively with sat. aq. $NaHCO_3$ (2 × 100 cm^3) and brine (50 cm^3), dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (30% (v/v) EtOAc in light petroleum) to give furanoside **8a** as a white solid material (11.6 g, 72% from **6a**). R_f 0.35 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26 (2H, d, *J* 8.6, Ar-H), 6.89 (2H, d, *J* 8.7, Ar-H), 4.81 (1H, s, H-1), 4.59 (1H, d, *J* 11.2, H-5a) 4.53–4.44 (3H, m, H-5b and CH_2 (MPM)), 4.10 and 4.09 (1H each, 2s, H-2 and H-3), 4.00 (1H, d, *J* 7.4, H-5′a), 3.81 (3H, s, OCH_3 (MPM)), 3.69 (1H, d, *J* 7.4, H-5′b), 3.37 (3H, s, OCH_3), 3.05 (3H, s, SO_2CH_3); δ_C ($CDCl_3$) 159.6, 129.5, 129.3 and 114.0 (Ar), 105.3 (C-1), 83.2 (C-4), 78.6 and 77.2 (C-2 and C-3), 72.1 (CH_2 (MPM)), 71.8(C-5′), 66.3(C-5), 55.6 (OCH_3), 55.4 (OCH_3 (MPM)), 37.8 (SO_2CH_3); MALDI-HRMS: *m*/*z* 397.0928 ([M + Na]$^+$, $C_{16}H_{22}O_8SNa^+$ calc. 397.0927). Similarly the crude mixture (~5.5 g) obtained from methanolic ammonia treatment of **6b** was reacted with sodium hydride (1.0 g, 60% suspension in mineral oil (w/w), 25 mmol)). After work-up, the residue was purified by column chromatography (45% (v/v) EtOAc in light petroleum) affording furanoside **8b** as a white solid material (2.54 g, 69% from **6b**). R_f 0.24 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.27 (2H, d, *J* 8.6, Ar-H), 6.89 (2H, d, *J* 9.0, Ar-H), 4.99 (1H, s, H-1), 4.61 (1H, d, *J* 11.7, CH_2 (MPM)), 4.52 (1H, d, *J* 11.4, CH_2 (MPM)), 4.48 (1H, d, *J* 11.9, H-5a), 4.41 (1H, d, *J* 11.6, H-5b), 4.18 (1H, s, H-2), 4.04 (1H, d, *J* 8.0, H-5′a), 3.92 (1H, d, *J* 8.8, H-5′b), 3.91 (1H, s, H-3), 3.81 (3H, s, OCH_3 (MPM)), 3.47 (3H, s, OCH_3), 3.05 (3H, s, SO_2CH_3); δ_C ($CDCl_3$) 159.5, 129.4, 128.8 and 113.9 (Ar), 104.2 (C-1), 86.2 (C-4), 79.2 (C-3), 76.9 (C-2), 72.1 (C-5′), 71.7 (CH_2 (MPM)), 66.0 (C-5), 56.2 (OCH_3), 55.2 (OCH_3 (MPM)), 37.5 (SO_2CH_3); MALDI-HRMS: *m*/*z* 397.0926 ([M + Na]$^+$ calc. $C_{16}H_{22}O_8$-SNa^+ 397.0927).

(1*S*,3*RS*,4*S*,7*R*)-1-Acetoxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (9). To a stirred solution of furanoside **8a** (11.6 g, 31.0 mmol) in anhydrous dioxane (45 cm^3) was added 18-crown-6 (16.4 g, 62.0 mmol) and KOAc (15.2 g, 154.9 mmol), and the mixture was heated under reflux for 12 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue dissolved in CH_2Cl_2 (100 cm^3). Washing was performed successively with sat. aq. $NaHCO_3$ (2 × 50 cm^3) and brine (50 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (20% (v/v) EtOAc in light petroleum) to afford furanoside **9a** as a white solid material (9.65 g, 92%). R_f 0.63 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26 (2H, d, *J* 7.9, Ar-H), 6.88 (2H, d, *J* 8.4, Ar-H), 4.80 (1H, s, H-1), 4.59 (1H, d, *J* 11.6, CH_2 (MPM)), 4.48 (1H, d, *J* 11.7, CH_2 (MPM)), 4.47 (1H, d, *J* 12.7, H-5a), 4.27 (1H, d, *J* 12.4, H-5b), 4.09 (1H, s, H-2), 4.05 (1H, s, H-3), 3.99 (1H, d, *J* 7.4, H-5′a), 3.80 (3H, s, OCH_3 (MPM)), 3.71 (1H, d, *J* 7.4, H-5′b), 3.36 (3H, s, OCH_3), 2.06 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 170.4 ($COCH_3$), 159.3, 129.4, 129.2 and 113.7 (Ar), 104.9 (C-1), 83.1 (C-4), 78.7 (C-2), 76.9 (C-3), 71.9 (CH_2 (MPM)), 71.8 (C-5′), 60.9 (C-5), 55.2 and 55.1 (OCH_3 and OCH_3 (MPM)), 20.6 ($COCH_3$); MALDI-HRMS: *m*/*z* 361.1245 ([M + Na]$^+$, $C_{17}H_{22}$-O_7Na^+ calc. 361.1258). Similarly, reaction of furanoside **8b** (646 mg, 1.73 mmol) with KOAc (880 mg, 8.97 mmol) in the presence of 18-crown-6 ether (940 mg, 3.56 mmol) in anhydrous dioxane (3 cm^3) followed by work-up and column chromatography (30% (v/v) EtOAc–light petroleum) afforded furanoside **9b** as a white solid material (455 mg, 78%). R_f 0.29 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26 (2H, d, *J* 9.0, Ar-H), 6.88 (2H, d, *J* 8.3, Ar-H), 4.98 (1H, s, H-1), 4.61 (1H, d, *J* 11.7, CH_2 (MPM)), 4.49 (1H, d, *J* 11.7, CH_2 (MPM)), 4.35 (1H, d, *J* 12.9, H-5a), 4.28 (1H, d, *J* 12.8, H-5b), 4.18 (1H, s, H-2), 4.02 (1H, d, *J* 8.0, H-5′a), 3.92 (1H, d, *J* 7.9, H-5′b), 3.87 (1H, s, H-3), 3.80 (3H, s, OCH_3 (MPM)), 3.48 (3H, s, OCH_3), 2.05 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 170.6 ($COCH_3$), 159.6, 129.5, 129.3 and 113.9 (Ar), 104.3 (C-1), 86.6 (C-4), 79.5 (C-3), 77.1 (C-2), 72.6 (C-5′), 71.7 (CH_2 (MPM)), 60.8 (C-5), 56.4 (OCH_3), 55.4 (OCH_3 (MPM)), 20.8 ($COCH_3$); MALDI-HRMS: *m*/*z* 361.1253 ([M + Na]$^+$, $C_{17}H_{22}O_7Na^+$ calc. 361.1258).

(1*R*,3*RS*,4*S*,7*R*)-1-Hydroxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (10). A solution of furanoside **9a** (2.27 g, 6.71 mmol) in sat. methanolic ammonia (55 cm^3) was stirred for 12 h at rt. The reaction mixture was evaporated to dryness under reduced pressure and coevaporated with toluene (2 × 10 cm^3). The residue was purified by column chromatography (55% (v/v) EtOAc in light petroleum) to give furanoside **10a** as a white solid material (1.91 g, 96%). R_f 0.31 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.28–7.25 (2H, m, Ar-H), 6.89–6.86 (2H, m, Ar-H), 4.80 (1H, s, H-1), 4.59 (1H, d, *J* 11.3, CH_2 (MPM)), 4.52 (1H, d, *J* 11.7, CH_2 (MPM)), 4.09 (2H, s, H-2 and H-3), 3.97 (1H, d, *J* 7.6, H-5′a), 3.86 (1H, s, H-5a), 3.84 (1H, s, H-5b), 3.79 (3H, s, OCH_3 (MPM)), 3.64 (1H, d, *J* 7.3, H-5′b), 3.37 (3H, s, OCH_3), 2.14 (1H, dd, *J* 6.0 and 6.6, 5-OH); δ_C ($CDCl_3$) 159.5, 129.8, 129.4 and 113.9 (Ar), 105.2 (C-1), 85.6 (C-4), 78.3 and 77.4 (C-2 and C-3), 71.9 (CH_2 (MPM)), 71.8 (C-5′), 58.8 (C-5), 55.5 and 55.3

(OCH_3 and OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 319.1140 ([M + Na]$^+$, $C_{15}H_{20}O_6Na^+$ calc. 319.1152). Similarly, reaction of furanoside **9b** (416 mg, 1.23 mmol) with sat. methanolic ammonia (20 cm^3) followed by work-up and column chromatography (60% (v/v) EtOAc in light petroleum) yielded furanoside **10b** as a white solid material (328 mg, 90%). R_f 0.36 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$) 7.28 (2H, d, *J* 7.3, Ar-H), 6.88 (2H, d, *J* 8.5, Ar-H), 4.97 (1H, s, H-1), 4.61 (1H, d, *J* 11.8, CH_2 (MPM)), 4.54 (1H, d, *J* 11.3, CH_2 (MPM)), 4.16 (1H, s, H-2), 3.98 (1H, d, *J* 8.0, H-5′a), 3.94 (1H, s, H-3), 3.86 (1H, d, *J* 7.8, H-5′b), 3.82–3.77 (5H, m, H-5 and OCH_3 (MPM)), 3.47 (3H, s, OCH_3), 2.11 (1H, t, *J* 6.0, 5-OH); δ_C ($CDCl_3$) 159.5, 129.5, 129.4 and 114.0 (Ar), 104.3 (C-1), 89.1 (C-4), 78.9 (C-3), 77.4 (C-2), 72.3 (C-5′), 71.8 (CH_2 (MPM)), 58.7 (C-5), 56.3 (OCH_3), 55.4 (OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 319.1149 ([M + Na]$^+$, $C_{15}H_{20}O_6Na^+$ calc. 319.1152).

(1*R*,3*SR*,4*S*,7*R*)-3-Methoxy-7-(*p*-methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (11). Sodium hydride (240 mg, 60% suspension in mineral oil, 6.00 mmol) was added to anhydrous THF (3 cm^3) and the resulting mixture was cooled to 0 °C. A solution of furanoside **10a** (667 mg, 2.25 mmol) in anhydrous THF (3 cm^3) was added dropwise. The temperature of the mixture was allowed to increase to rt and stirring was continued for 15 min. *p*-Methoxybenzyl chloride (463 mg, 2.96 mmol) was added dropwise followed by tetrabutylammonium iodide (554 mg, 1.5 mmol), and the resulting mixture was stirred for 36 h at rt. The mixture was cooled to 0 °C and ice-cold H_2O (10 cm^3) was carefully added. Extraction was performed with EtOAc (2 × 20 cm^3) and the combined organic phase was washed with brine (20 cm^3), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (35% EtOAc in light petroleum, containing 1% Et_3N, v/v/v) to afford furanoside **11a** as a colourless oil (806 mg, 86%). R_f 0.62 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26–7.20 (4H, m, Ar-H), 6.88–6.84 (4H, m, Ar-H), 4.79 (1H, s, H-1), 4.56 (1H, d, *J* 11.6, CH_2 (MPM)), 4.53 (2H, s, CH_2 (MPM)), 4.47 (1H, d, *J* 11.5, CH_2 (MPM)), 4.07 and 4.04 (1H each, 2s, H-2 and H-3), 3.95 (1H, d, *J* 7.2, H-5′a), 3.79 (6H, s, 2 × OCH_3 (MPM)), 3.75 (1H, d, *J* 7.8, H-5′b), 3.72 (1H, s, H-5a), 3.71 (1H, s, H-5b), 3.38 (3H, s, OCH_3); δ_C ($CDCl_3$) 159.4, 159.3, 130.1, 129.8, 129.4, 129.3, 113.9 and 113.8 (Ar) 105.0 (C-1), 85.2 (C-4), 78.9 and 77.3 (C-2 and C-3), 73.4 (CH_2 (MPM)), 72.4 (C-5′), 71.9 (CH_2 (MPM)), 66.3 (C-5), 55.5 (OCH_3), 55.3 (2 × OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 439.1726 ([M + Na]$^+$, $C_{23}H_{28}O_7Na^+$ calc. 439.1727). Similarly, furanoside **10b** (278 mg, 0.94 mmol) was reacted with *p*-methoxybenzyl chloride (157 mg, 1.00 mmol) in the presence of NaH (102 mg, 60% suspension in mineral oil, 2.55 mmol), tetrabutylammonium iodide (185 mg, 0.5 mmol) and anhydrous THF (2 cm^3) followed by work-up and column chromatography (50% EtOAc in light petroleum, containing 1% Et_3N, v/v/v) to give furanoside **11b** as a colourless oil (316 mg, 81%). R_f 0.31 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.26–7.20 (4H, m, Ar-H), 6.87–6.85 (4H, m, Ar-H), 4.99 (1H, s, H-1), 4.60–4.46 (4H, m, 2 × CH_2 (MPM)), 4.13 (1H, s, H-2), 3.95–3.87 (3H, m, H-3 and H-5′), 3.79 (6H, s, 2 × OCH_3 (MPM)), 3.65 (2H, s, H-5), 3.48 (3H, s, OCH_3); δ_C ($CDCl_3$) 159.5, 159.4, 129.9, 129.6, 129.5, 129.4, 113.9 and 113.8 (Ar), 104.1 (C-1), 88.6 (C-4), 79.3 (C-3), 77.3 (C-2), 73.4 (2 × CH_2 (MPM)), 72.7 (C-5′), 71.7 (CH_2 (MPM)), 65.4 (C-5), 56.4 (OCH_3), 55.3 (OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 439.1713 ([M + Na]$^+$, $C_{23}H_{28}O_7Na^+$ calc. 439.1727.

(2*S*,3*R*,4*R*)-4-Hydroxy-3-(*p*-methoxybenzyloxy)-4-(*p*-methoxybenzyloxymethyl)tetrahydrofuran-2-carbaldehyde (12). A solution of furanoside **11a** (7.19 g, 17.3 mmol) in 70% aqueous acetic acid (100 cm^3 (v/v)) was stirred for 12 h at rt. The mixture was evaporated to dryness under reduced pressure and the residue was successively coevaporated with absolute ethanol (3 × 50 cm^3) and toluene (3 × 50 cm^3) and purified by column chromatography (40 (v/v) EtOAc in light petroleum) to give aldehyde **12** as a white solid material (5.90 g, 85%). R_f 0.33 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 9.63 (1H, s, H-1), 7.18 (4H, d, *J* 8.5, Ar-H), 6.86 (2H, d, *J* 8.5, Ar-H), 6.85 (2H, d, *J* 8.7, Ar-H), 4.58 (1H, d, *J* 11.2, CH_2 (MPM)), 4.49 (1H, d, *J* 11.4, CH_2 (MPM)), 4.44 (1H, d, *J* 11.5, CH_2 (MPM)), 4.43 (1H, d, *J* 11.4, CH_2 (MPM)), 4.35 (1H, s, H-2), 3.94 (1H, d, *J* 9.2, H-5′a), 3.90 (1H, s, H-3), 3.88 (1H, d, *J* 9.1, H-5′b), 3.79–3.75 (7H, m, 2 × OCH_3 and H-5a), 3.44 (1H, d, *J* 9.3, H-5b), 2.80 (1H, s, 4-OH); δ_C ($CDCl_3$) 203.6 (C-1), 159.5, 159.4, 129.6, 129.5, 129.3, 114.0 and 113.9 (Ar), 87.3 (C-2), 86.8 (C-3), 81.0 (C-4), 75.1 (C-5′), 73.4 (CH_2 (MPM)), 71.6 (CH_2 (MPM)), 67.6 (C-5), 55.3 (2 × OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 425.1570 ([M + Na]$^+$, $C_{22}H_{26}O_7Na^+$ calc. 425.1570. Similarly, reaction of furanoside **11b** (3.19 g, 7.66 mmol) with 70% aqueous acetic acid (50 cm^3) followed by evaporation, coevaporation and column chromatography afforded aldehyde **12** (2.47 g, 80%) with analytical data identical to those listed above.

(2*R*,3*R*,4*R*)-4-Hydroxy-2-[(*S*)-hydroxy(phenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (13a). Phenyl magnesium bromide (1.0 M solution in THF, 40 cm^3, 40 mmol) was added dropwise during 15 min to a stirred solution of aldehyde **12** (2.90 g, 7.21 mmol) in anhydrous THF (25 cm^3) at 0 °C. The temperature was allowed to rise to rt and stirring was continued for 12 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue diluted with CH_2Cl_2 (50 cm^3) and washed with sat. aq. NH_4Cl (3 × 25 cm^3). The organic phase was dried (Na_2SO_4), filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (30% (v/v) EtOAc in light petroleum) affording tetrahydrofuran **13a** (2.57 g, 74%) as a white solid material. R_f 0.33 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 7.40–7.29 (5H, m), 7.21 (2H, d, *J* 8.8), 6.90–6.84 (4H, m), 6.76 (2H, d, *J* 8.7), 4.73 (1H, dd, *J* 2.6 and 6.3, H-1′), 4.48 (2H, s, CH_2 (MPM)), 4.09–4.05 (3H, m, H-2′ and CH_2 (MPM)), 3.88 (1H, d, *J* 9.3, H-5″a), 3.81–3.75 (7H, m, 2 × OCH_3 and H-5″b), 3.73–3.69 (2H, m, H-3′ and H-5′a), 3.49 (1H, d, *J* 9.4, H-5′b), 3.36 (1H, d, *J* 2.5, 1′-OH), 3.21 (1H, s, 4′-OH); δ_C ($CDCl_3$) 159.5, 159.4, 140.7, 129.7, 129.6, 129.5, 128.6, 128.1, 127.3, 114.0 and 113.8 (Ar), 89.4 (C-2′), 84.6 (C-3′), 81.9 (C-4′), 75.4 (C-5″), 74.8 (C-1′), 73.5 (CH_2 (MPM)), 71.7 (CH_2 (MPM)), 69.3 (C-5′), 55.4 (2 × OCH_3); MALDI-HRMS: *m*/*z* 503.2016 ([M + Na]$^+$, $C_{28}H_{32}O_7Na^+$ calc. 503.2040).

(2*R*,3*R*,4*R*)-4-Hydroxy-2-[(*S*)-hydroxy(1-pyrenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (13b). 1-Bromopyrene (6.27 g, 22.3 mmol) was added to a stirred mixture of magnesium turnings (930 mg, 38.3 mmol) and iodine (20 mg) in THF (20 cm^3). The mixture was stirred at 40 °C for 1 h whereupon it was cooled to rt. A solution of aldehyde **12** (3.03 g, 7.53 mmol) in THF (10 cm^3) was added slowly and the reaction mixture was stirred for 12 h. The crude product obtained after work-up (as described above for **13a**) was purified by column chromatography (40% (v/v) EtOAc in light petroleum) to give tetrahydrofuran **13b** (3.25 g, 71%) as a pale yellow solid. R_f 0.43 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 8.33–8.27 (2H, m), 8.20–8.13 (3H, m), 8.05–7.99 (4H, m), 7.15 (2H, d, *J* 8.6), 6.82 (2H, d, *J* 8.7), 6.30 (2H, d, *J* 8.7), 6.20 (2H, d, *J* 8.3), 5.87 (1H, d, *J* 6.7, H-1′), 4.43 (2H, s, CH_2 (MPM)), 4.41 (1H, m, H-2′), 4.01 (1H, d, *J* 9.3, H-5″a), 3.91 (1H, d, *J* 11.9, CH_2 (MPM)), 3.86 (1H, d, *J* 9.4, H-5″b), 3.77 (3H, s, OCH_3), 3.71–3.64 (4H, m, H-3′, H-5′a, OH, and CH_2 (MPM)), 3.50 (1H, d, *J* 9.4, H-5′b), 3.44 (3H, s, OCH_3), 3.32 (1H, br s, OH); δ_C ($CDCl_3$) 159.5, 158.9, 133.9, 131.4, 131.1, 130.7, 129.8, 129.7, 129.5, 129.2, 128.9, 128.5, 127.8,

127.7, 127.5, 126.0, 125.5, 125.3, 125.2, 125.1, 125.0, 124.9, 122.9, 113.9 and 113.3 (Ar), 89.5 (C-2′), 83.5 (C-3′), 82.0 (C-4′), 75.7 (C-5″), 73.4 (CH_2 (MPM)), 71.3 and 71.0 (C-1′ and CH_2 (MPM)), 69.3 (C-5′), 55.3 (OCH_3), 55.0 (OCH_3); MALDI-HRMS: *m*/*z* 627.2343 ([M + Na]$^+$, $C_{38}H_{36}O_7Na^+$ calc. 627.2353).

(2*S*,3*R*,4*R*)-4-Hydroxy-2-[phenylcarbonyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (14a). Dess Martin periodinane (2.01 g, 4.74 mmol) was added to the solution of compound **13a** (1.08 g, 2.25 mmol) in CH_2Cl_2 (10 cm^3) and the reaction mixture was stirred for 12 h at rt. Diethyl ether (100 cm^3) was added and the resulting mixture was poured into sat. aq. $NaHCO_3$ (100 cm^3, containing 2 g of $Na_2S_2O_3$). The resulting mixture was slowly stirred until the white solid material dissolved. The phases were separated, and the aqueous layer was washed with ether (50 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (25% (v/v) EtOAc in light petroleum) to give compound **14a** as a white solid material (823 mg, 77%). R_f 0.52 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.91–7.88 (2H, m), 7.57–7.54 (1H, m), 7.41–7.36 (2H, m), 7.25–7.16 (4H, m) and 6.88–6.82 (4H, m, Ar-H), 5.14 (1H, d, *J* 2.3, H-2′), 4.59 (1H, d, *J* 11.2, CH_2 (MPM)), 4.52–4.49 (3H, m, CH_2 (MPM)), 4.25 (1H, d, *J* 2.3, H-3′), 3.97 (2H, s, H-5″), 3.79–3.77 (7H, m, H-5′a and 2 × OCH_3), 3.57 (1H, d, *J* 9.4, H-5′b); δ_C ($CDCl_3$) 198.2 (C-1′), 159.5, 159.4, 135.5, 133.3, 129.8, 129.7, 129.6, 129.0, 128.5 and 114.0 (Ar), 86.7 (C-2′), 86.2 (C-3′), 81.9 (C-4′), 75.6 (C-5″), 73.5 and 72.7 (CH_2 (MPM)), 69.0 (C-5′), 55.3 (2 × OCH_3); MALDI-HRMS: *m*/*z* 501.1868 ([M + Na]$^+$, $C_{28}H_{30}O_7Na^+$ calc. 501.1884).

(2*S*,3*R*,4*R*)-4-Hydroxy-2-[1-pyrenylcarbonyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (14b). 3Å Molecular sieve powder (3.0 g) was activated by heating at 200 °C for 2 h under reduced pressure. After cooling to rt under an atmosphere of N_2, pyridinium dichromate (1.5 g, 3.99 mmol) and anhydrous CH_2Cl_2 (20 cm^3, freshly filtered through basic alumina) were added. Compound **13b** (1.51 g, 2.50 mmol) was added in one portion to the stirred solution and the sides of the flask were washed with CH_2Cl_2 (5 cm^3). The resulting mixture was stirred for 2 h at rt and then filtered through 3Å molecular sieve powder on a glass filter. The plug was washed with CH_2Cl_2 (3 × 10 cm^3) and the combined filtrate concentrated to dryness under reduced pressure. The resulting brownish residue was suspended in ethyl acetate (50 cm^3), sonicated for 5 min, filtered through 3Å molecular sieve powder, washed with ethyl acetate (2 × 20 cm^3) and concentrated to dryness under reduced pressure to give a residue which was used in the next step without further purification.

(2*R*,3*R*,4*R*)-4-Hydroxy-2-[(*R*)-hydroxy(phenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (15a). $NaBH_4$ (330 mg, 8.71 mmol) was added at 0 °C to the solution of compound **14a** (1.93 g, 4.03 mmol) in a mixture of THF (10 cm^3) and H_2O (2.0 cm^3). The resulting mixture was stirred at rt for 30 min whereupon ethyl acetate (50 cm^3) and H_2O (25 cm^3) were added. The phases were separated and the organic phase was washed with sat. aqueous $NaHCO_3$ (2 × 25 cm^3), dried (Na_2SO_4), filtered and then concentrated to dryness under reduced pressure. The residue was purified by column chromatography (25% (v/v) EtOAc in light petroleum) to give compound **15a** (432 mg, 22%) as a white solid material while elution with 30–35% (v/v) EtOAc in light petroleum afforded stereoisomer **13a** (1.04 g, 54%). Data for **15a**: R_f 0.73 (EtOAc–light petroleum 50 : 50, v/v);δ_H ($CDCl_3$) 7.45–7.43 (2H, m), 7.39–7.35 (2H, m), 7.31–7.25 (1H, m), 7.22–7.19 (2H, m), 6.86–6.83 (2H, m), 6.75–6.72 (4H, m), 5.08 (1H, d, *J* 2.3, H-1′), 4.48 (2H, s, CH_2 (MPM)), 4.17 (1H, dd, *J* 2.6 and 2.7, H-2′), 3.91 (1H, d, *J* 9.4, H-5′a), 3.83 (1H, s, OH), 3.78 (4H, s, OCH_3 and H-3′), 3.76 (3H, s, OCH_3), 3.75 (1H, d, *J* 9.2, H-5′b), 3.70 (1H, d, *J* 9.5, H-5″a), 3.68 (1H, d, *J* 10.9, CH_2 (MPM)), 3.63 (1H, d, *J* 11.3, CH_2 (MPM)), 3.58 (1H, s, OH), 3.51 (1H, d, *J* 9.7, H-5″b); δ_C ($CDCl_3$) 159.5, 159.2, 140.7, 129.8, 129.6, 129.2, 128.6, 127.6, 126.1, 114.0 and 113.7 (Ar), 90.9 (C-2′), 82.1 (C-3′), 81.2 (C-4′), 75.3 (CH_2 (MPM)), 73.6 (CH_2 (MPM)), 73.1 (C-1′), 71.1 (C-5″), 69.0 (C-5′), 55.3 (OCH_3 (MPM)); MALDI-HRMS: *m*/*z* 503.2018 ([M + Na]$^+$, $C_{28}H_{32}O_7Na^+$ calc. 503.2040).

(2*R*,3*R*,4*R*)-4-Hydroxy-2-[(*R*)-hydroxy(1-pyrenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (15b). The crude **14b** (~1.4 g) was reacted with $NaBH_4$ (166 mg, 4.49 mmol) in a 1 : 4 mixture of H_2O–THF (10 cm^3). Work-up (as described above for **15a**) followed by column chromatography (30–35% (v/v) EtOAc in light petroleum) furnished compound **15b** (246 mg, 16% from **13b**, two steps) as a white solid material while elution with 35–40% (v/v) EtOAc in light petroleum furnished the stereoisomer **13b** (507 mg, 34%) as a white solid material. Data for **15b**: R_f 0.53 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 8.27 (1H, *J* 8.3), 8.08–8.02 (4H, m), 7.93–7.86 (4H, m), 7.16–7.10 (2H, m), 6.76–6.73 (2H, m), 6.14–6.11 (2H, m), 5.95 (3H, d, *J* 8.4, Ar-H and H-1′), 4.42–4.39 (3H, m, H-2′ and CH_2 (MPM)), 4.29 (1H, br s, OH), 3.93 (1H, d, *J* 9.3, H-5″a), 3.83–3.60 (7H, m, H-3′, H-5″b, OH, OCH_3 and CH_2 (MPM)), 3.45–3.42 (4H, m, OCH_3 and CH_2 (MPM)), 3.22 (1H, d, *J* 11.7, H-5′a), 3.09 (1H, d, *J* 11.6, H-5′b); δ_C ($CDCl_3$) 159.4, 158.7, 133.6, 131.3, 130.8, 130.6, 129.7, 129.6, 129.1, 128.5, 127.9, 127.5, 127.3, 127.2, 126.0, 125.4, 125.2, 124.8, 124.6, 123.6, 121.9, 113.9 and 113.1 (Ar), 89.4 (C-2′), 82.7 (C-3′), 81.3 (C-4′), 75.4 (C-5″), 73.5 (CH_2 (MPM)), 70.8 and 70.6 (C-1′ and CH_2 (MPM)), 69.0 (C-5′), 55.3 (OCH_3), 55.0 (OCH_3); MALDI-HRMS: *m*/*z* 627.2329 ([M + Na]$^+$, $C_{38}H_{36}O_7Na^+$ calc. 627.2353).

(1*R*,3*S*,4*S*,7*R*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (16a). *N*,*N*,*N*′,*N*′-Tetramethylazodicarboxamide (TMAD, 277 mg, 1.61 mmol) was added in one portion to a stirred solution of compound **15a** (492 mg, 1.02 mmol) and tributylphosphine (PBu_3, 328 mg, 1.62 mmol) in anhydrous benzene (10 cm^3) at 0 °C. The mixture was stirred 12 h at rt and was then diluted with diethyl ether (50 cm^3). Washing was performed successively with sat. aq. NH_4Cl (2 × 20 cm^3) and brine (25 cm^3) and the separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (15–20% (v/v) EtOAc in light petroleum) to give compound **16a** as a white solid material (461 mg, 97%). R_f 0.75 (EtOAc–light petroleum 50 : 50, v/v); δ_H ($CDCl_3$) 7.36–7.34 (4H, m), 7.30–7.24 (5H, m), 6.89–6.86 (4H, m), 5.15 (1H, s, H-1′), 4.63 (1H, d, *J* 11.8, CH_2 (MPM)), 4.62 (1H, d, *J* 12.1, CH_2 (MPM)), 4.56 (2H, d, *J* 11.4, CH_2 (MPM)), 4.34 and 4.28 (1H each, 2s, H-2′ and H-3′), 4.00 (1H, d, *J* 7.6, H-5″a), 3.94 (1H, d, *J* 7.8, H-5″b), 3.80 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (2H, s, H-5′); δ_C ($CDCl_3$) 159.5, 159.4, 138.7, 130.1, 129.9, 129.5, 129.4, 128.2, 127.5, 126.1, 113.9 and 113.8 (Ar), 87.4 (C-4′), 82.2 (C-1′), 80.8 and 79.4 (C-2′ and C-3′), 73.9 (C-5″), 73.5 (CH_2 (MPM)), 71.7 (CH_2 (MPM)), 65.9 (C-5′), 55.4 (2 × OCH_3); MALDI-HRMS: *m*/*z* 485.1921 ([M + Na]$^+$, $C_{28}H_{30}O_6Na^+$ calc. 485.1935).

(1*R*,3*S*,4*S*,7*R*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-(1-pyrenyl)-2,5-dioxabicyclo[2.2.1]heptane (16b). Reaction of compound **15b** (401 mg, 0.66 mmol) in the presence of TMAD (170 mg, 0.98 mmol), PBu_3 (200 mg, 0.99 mmol) and benzene (10 cm^3) followed by work-up (as described above for **16a**) and column chromatography (20% (v/v) EtOAc in light petroleum) afforded compound **16b** as a pale yellow solid (342 mg, 88%). R_f 0.53 (EtOAc–light petroleum 50 : 50,

v/v); δ_H ($CDCl_3$) 8.42 (1H, d, *J* 8.0), 8.21 (1H, d, *J* 7.9), 8.15 (2H, d, *J* 7.9), 8.07 (1H, d, *J* 9.2), 8.04–7.95 (4H, m), 7.33 (2H, d, *J* 8.5), 7.29 (2H, d, *J* 8.4), 6.90 (2H, d, *J* 8.7), 6.89 (2H, d, *J* 8.4), 6.17 (1H, s, H-1′), 4.72 (1H, s, H-2′), 4.71–4.61 (4H, m, 2 × CH_2 (MPM)), 4.52 (1H, s, H-3′), 4.21 (1H, d, *J* 7.6, H-5″a), 4.07 (1H, d, *J* 7.8, H-5″b), 3.93 (1H, d, *J* 11.4, H-5′a), 3.88 (1H, d, *J* 11.4, H-5′b), 3.80 (6H, s, 2 × OCH_3); δ_C ($CDCl_3$) 159.5, 159.4, 131.9, 131.4, 130.9, 130.6, 130.2, 129.9, 129.6, 129.5, 129.4, 129.3, 127.8, 127.7, 127.6, 127.4, 127.1, 125.9, 125.4, 125.1, 124.9, 124.8, 124.2, 121.9, 114.0 and 113.9 (Ar), 87.5 (C-4′), 81.1 (C-3′), 80.6 (C-1′), 79.3 (C-2′), 73.8 (C-5″), 73.6 (CH_2 (MPM)), 71.9 (CH_2 (MPM)), 66.1 (C-5′), 55.4 (2 × OCH_3); MALDI-HRMS: *m*/*z* 609.2240 ($[M + Na]^+$, $C_{38}H_{34}O_6Na^+$ calc. 609.2247).

(1*R*,3*S*,4*S*,7*R*)-7-Hydroxy-1-hydroxymethyl-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (17a). 2,3-Dichloro-5,6-dicyanoquinone (DDQ, 417 mg, 1.84 mmol) was added at rt to a stirred solution of compound **16a** (461 mg, 1.0 mmol) in CH_2Cl_2 (7 cm^3) containing a small amount of H_2O (0.3 cm^3). Immediate appearance of a deep greenish-black colour which slowly faded into pale brownish-yellow was observed. The reaction mixture was vigorously stirred for 4 h. The precipitate was removed by filtration through a short pad of silica gel which was washed with EtOAc (2 × 25 cm^3). The combined filtrate was washed successively with sat. aq. $NaHCO_3$ (25 cm^3) and brine (25 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (65% (v/v) EtOAc in light petroleum) to give compound **17a** as a white solid material (140 mg, 63%). R_f 0.38 (EtOAc–light petroleum 75 : 25, v/v); δ_H ($CDCl_3$–CD_3OD; 5 : 1) 7.30–7.21 (5H, m, Ar-H), 5.12 (1H, s, H-1′), 4.38 (1H, s, H-2′), 4.20 (1H, s, H-3′), 3.91 (1H, d, *J* 8.1, H-5″a), 3.83–3.80 (3H, m, H-5′ and H-5″b), 2.99 (1H, s, OH); δ_C ($CDCl_3$–CD_3OD, 5 : 1) 138.4, 128.3, 127.5, 125.8, 88.3, 81.9, 81.8, 74.4, 72.7, 58.2; MALDI-HRMS: *m*/*z* 245.0791 ($[M + Na]^+$, $C_{12}H_{14}O_4Na^+$ calc. 245.0784).

(1*R*,3*S*,4*S*,7*R*)-7-Hydroxy-1-hydroxymethyl-3-(1-pyrenyl)-2,5-dioxabicyclo[2.2.1]heptane (17b). Reaction of compound **16b** (326 mg, 0.57 mmol) with DDQ (452 mg, 1.99 mmol) in a mixture of CH_2Cl_2 (8 cm^3) and H_2O (0.5 cm^3) at rt followed by work-up (as described above for **17a**) and column chromatography (2% (v/v) MeOH in CH_2Cl_2) furnished compound **17b** as a white solid material (100 mg, 52%). R_f 0.34 (EtOAc–light petroleum 75 : 25 v/v); δ_H ($(CD_3)_2SO$), 8.43 (1H, d, *J* 8.1), 8.34–8.29 (4H, m), 8.22 (1H, d, *J* 9.5), 8.17–8.08 (3H, m), 6.22 (1H, s, H-1′), 5.68 (1H, d, *J* 4.4, 3′-OH), 4.96 (1H, dd, *J* 5.5 and 6.1, 5′-OH), 4.54 (1H, d, *J* 4.8, H-3′), 4.52 (1H, s, H-2′), 4.03 (1H, d, *J* 7.8, H-5″a), 3.91 (1H, d, *J* 7.7, H-5″b), 3.88 and 3.86 (1H each, 2s, H-5′); δ_C ($(CD_3)_2SO$) 133.7, 130.8, 130.1, 129.9, 127.4, 127.3, 126.8, 126.1, 125.2, 125.1, 124.4, 124.2, 124.0, 123.7 and 122.6 (Ar), 88.5 (C-4′), 81.7 (C-2′), 79.2 (C-1′), 74.2 (C-3′), 72.4 (C-5″), 58.2 (C-5′); MALDI-HRMS: *m*/*z* 369.1106 ($[M + Na]^+$, $C_{22}H_{18}O_4Na^+$ calc. 369.1097).

(1*S*,3*S*,4*S*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (18a). 4,4′-Dimethoxytrityl chloride (195 mg, 0.58 mmol) was added in one portion to a stirred solution of the compound **17a** (101 mg, 0.45 mmol) in anhydrous pyridine (1.5 cm^3). After stirring for 12 h at rt, methanol (0.1 cm^3) was added and the resulting mixture was concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 cm^3) and washed with sat. aq. $NaHCO_3$ (2 × 10 cm^3). The separated organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and coevaporated with toluene (2 × 2 cm^3). The residue was purified by column chromatography (50% ethyl acetate in light petroleum, containing 0.5% Et_3N (v/v/v)) to afford compound **18a** as a white solid material (210 mg, 88%). R_f 0.59 (EtOAc–light petroleum 75 : 25 v/v); δ_H ($CDCl_3$) 7.39 (2H, d, *J* 7.5), 7.38–7.15 (12H, m), 6.77 (4H, d, *J* 8.9), 5.15 (1H, s, H-1′), 4.34 (1H, d, *J* 5.1, H-3′), 4.21 (1H, s, H-2′), 3.94 (2H, s, H-5″), 3.71 (6H, s, 2 × OCH_3), 3.48 (1H, d, *J* 10.6, H-5′a), 3.42 (1H, d, *J* 10.4, H-5′b), 2.30 (1H, d, *J* 5.6, 3′-OH); δ_C ($CDCl_3$) 158.7, 144.6, 138.7, 135.8, 135.6, 130.2, 128.4, 128.2, 128.1, 127.5, 127.0, 125.9 and 113.4 (Ar), 86.7 and 86.6 (C-4′ and –CAr_3), 81.9 and 81.8 (C-1′ and C-2′), 76.4 (C-3′), 73.2 (C-5″), 61.3 (C-5′), 55.3 (2 × OCH_3), MALDI-HRMS: *m*/*z* 547.2095 ($[M + Na]^+$, $C_{33}H_{32}O_6Na^+$ calc. 547.2091).

(1*S*,3*S*,4*S*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(1-pyrenyl)-2,5-dioxabicyclo[2.2.1]heptane (18b). Reaction of compound **17b** (57 mg, 0.16 mmol) with DMTCl (64 mg, 0.19 mmol) in anhydrous pyridine (1 cm^3) followed by work-up (as described above for **18a**) and column chromatography (35% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)) afforded compound **18b** as a yellowish solid material (58 mg, 54%). R_f 0.72 (EtOAc–light petroleum 75:25, v/v); δ_H ($CDCl_3$) 8.38 (1H, d, *J* 8.0), 8.16 (1H, d, *J* 8.0), 8.08 (2H, d, *J* 8.1), 8.00–7.90 (5H, m), 7.45 (2H, d, *J* 8.5), 7.34 (4H, d, *J* 7.1), 7.32–7.16 (3H, m), 6.79 (4H, d, *J* 8.9), 6.11 (1H, s, H-1′), 4.64 (1H, s, H-2′), 4.57 (1H, br s, H-3′), 4.20 (1H, d, *J* 8.4, H-5″a), 4.01 (1H, d, *J* 8.4, H-5″b), 3.71 (6H, s, 2 × OCH_3), 3.60 (1H, d, *J* 10.4, H-5′a), 3.54 (1H, d, *J* 10.0, H-5′b), 2.42 (1H, br s, 3′-OH); δ_C ($CDCl_3$) 158.7, 144.7, 135.8, 135.7, 131.7, 131.4, 131.0, 130.6, 130.2, 128.2, 128.1, 127.7, 127.6, 127.5, 127.2, 127.0, 126.0, 125.7, 125.4, 125.2, 125.0, 124.9, 124.8, 123.9, 122.0 and 113.4 (Ar), 86.9 (C-4′), 86.6 (CAr_3), 81.8 (C-2′), 80.3 (C-1′), 76.6 (C-3′), 73.0 (C-5″), 61.4 (C-5′), 55.3 (2 × OCH_3); ESI-MS: *m*/*z* 671.5 $[M + Na]^{\bullet}$

(1*S*,3*S*,4*S*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)-phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (19a). 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (50 mg, 0.21 mmol) was added dropwise to a stirred solution of the compound **18a** (100 mg, 0.19 mmol) and *N*,*N*-diisopropylethylamine (0.2 cm^3) in anhydrous CH_2Cl_2 (1.0 cm^3) at rt. After stirring for 6 h at rt, the reaction mixture was diluted with EtOAc (20 cm^3, containing 0.5% Et_3N, v/v). Washing was performed with sat. aq. $NaHCO_3$ (2 × 10 cm^3) and the separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (20% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)) to give phosphoramidite **19a** as a white solid material (78 mg, 57%). R_f 0.61 (EtOAc–light petroleum 75 : 25, v/v); δ_P ($CDCl_3$) 149.2.

(1*S*,3*S*,4*S*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)-phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(1-pyrenyl)-2,5-dioxabicyclo[2.2.1]heptane (19b). Reaction of compound **18b** (65 mg, 0.1 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (29 mg, 0.12 mmol) in the presence of *N*,*N*-diisopropylethylamine (0.1 cm^3) and anhydrous CH_2Cl_2 (1.0 cm^3) followed by work-up (as described above for the compound **19a**) and column chromatography (25% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)) afforded phosphoramidite **19b** as a white solid material (36 mg, 42 %). R_f 0.75 (EtOAc–light petroleum 50:50 v/v); δ_P ($CDCl_3$) 149.3, 149.2.

Synthesis, deprotection and purification of oligonucleotides

All oligonucleotides were prepared using the phosphoramidite approach on a Biosearch 8750 DNA synthesizer in 0.2 μmol scale on CPG solid supports (BioGenex). The stepwise coupling efficiencies for phosphoramidites **19a** and **19b** (10 min coupling time) and for unmodified deoxynucleoside phosphoramidites (with standard 2 min coupling time) were >99%

using 1*H*-tetrazole as activator (pyridine hydrochloride was used as activator when coupling **19b**). After standard deprotection and cleavage from the solid support using 32% aqueous ammonia (12 h, 55 °C), the oligonucleotides were purified by precipitation from ethanol. The composition of the oligomers was verified by MALDI-MS analysis and the purity (>80%) by capillary gel electrophoresis. MALDI-MS *m*/*z* ($[M - H]^-$, found/calc.): **ON3**, 2732/2733; **ON4**, 2854/2857; **ON5**, 2789/2789; **ON6**, 2909/2913.

Thermal denaturation studies

The thermal denaturation experiments were performed on a Perkin-Elmer UV/VIS spectrometer fitted with a PTP-6 Peltier temperature-programming element using a medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) or a high salt buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) and concentrations of 1.0 μM of the two complementary strands (assuming identical extinction coefficients of the thyminyl, phenyl and pyrenyl moieties at 260 nm). During melting experiemnts, the absorbance was monitored at 260 nm while the temperature was raised at a rate of 1 °C min^{-1}. The melting temperatures (T_m values) of the duplexes were determined as the maximum of the first derivatives of the melting curves obtained.

Fluorescence measurements

Fluorescence emission intensities were recorded in buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5×10^{-7} M concentrations of each strand (assuming identical extinction coefficients of the thyminyl, phenyl and pyrenyl moieties at 260 nm). All measurements were carried out with argon-saturated solutions at 19 ± 1 °C. Duplexes were heated to 70 °C and cooled to 0 °C before degassing and fluorescence measurements. Excitation wavelength (λ_{ex}) was 280 nm. Emission slit width and excitation slit width were 4.0. Fluorescence intensities of band I (I_I) and band III (I_{III}) were measured at 383 nm and 401 nm, respectively.

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