Conformationally locked aryl *C*-nucleosides: synthesis of phosphoramidite monomers and incorporation into single-stranded DNA and LNA (locked nucleic acid)[1]

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Synthesis of a series of LNA-type β-configured *C*-aryl nucleosides, *i.e.*, 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl derivatives containing phenyl, 4-fluoro-3-methylphenyl, 1-naphthyl, 1-pyrenyl and 2,4,5-trimethylphenyl groups as aglycons, has been accomplished. The key synthetic step consisted of stereoselective Grignard reactions of the cyclic aldehyde **11** followed by cyclization to give the bicyclic core structure with a locked *N*-type furanose conformation as confirmed by NOE experiments on the di-*O*-*p*-methoxybenzyl derivatives **13a**–**13e** and an X-ray crystallographic study of the phenyl derivative **14a**. The phosphoramidite approach was used for automated incorporation of the LNA-type β-configured *C*-aryl monomers **17a**–**17e** into short DNA and 2′-OMe-RNA/LNA strands. It is shown that universal hybridization can be obtained with a conformationally restricted monomer as demonstrated most convincingly for the pyrene LNA monomer **17d**, both in a DNA context and in an RNA-like context. Increased binding affinity of oligonucleotide probes for universal hybridization can be induced by combining the pyrene LNA monomer **17d** with affinity-enhancing 2′-OMe-RNA/LNA monomers.

Introduction

The development of chemically modified nucleotide monomers for universal nucleic acid hybridization, *i.e.*, so-called universal bases able to bind isoenergetically with each of the natural nucleotides, is a research area of much current interest aiming at the development of primers for degenerate PCR reactions or universal hybridization probes.[2] Promising universal bases based on a 2-deoxy-β-D-ribofuranosyl moiety reported in the literature include derivatives of 3-nitropyrrole,[3] 5-nitroindole,[4] pyrene,[5] isocarbostyril[6] and 8-aza-7-deazaadenine.[7] Whereas incorporation of one of these DNA-type monomers into an oligodeoxynucleotide induced the desired minor variation in duplex melting temperature (T_m value) when placed opposite the four natural DNA bases, drawbacks are the concomitant decreased thermal stabilities obtained (ΔT_m values of typically −4 to −10 °C per universal base incorporated compared to the corresponding fully complementary reference DNA:DNA duplex).[2–8] Stimulated by the work of Kool and collaborators on hybridization using non-polar aromatic moieties as replacements of the natural bases[5,9] and the desire to obtain improved binding affinity for universal hybridization,[2] we became interested in studying LNA-type derivatives of aryl *C*-nucleosides containing various planar aromatic moieties as aglycons (Fig. 1). LNA (locked nucleic acid, Fig. 1),[10–13] defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomer(s),[10] is characterized by very high binding affinity and efficient Watson–Crick discrimination when hybridized with single stranded DNA or RNA targets.[10–15] Similar LNA-type *C*-nucleoside derivatives with various heterocyclic moieties as aglycons have been prepared and studied in triplex forming oligonucleotides.[16–18]

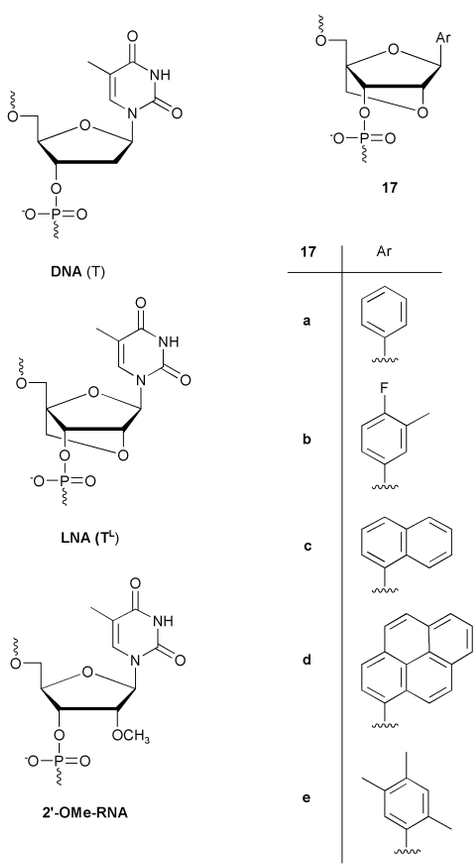

Fig. 1 Structures of nucleotide monomers studied: DNA (**T**), LNA ($\mathbf{T^L}$), 2′-OMe-RNA and LNA-type aryl *C*-nucleotides (**17a**–**17e**). The short notations shown are used in Table 1. For DNA, LNA and 2′-OMe-RNA, the thymine monomers are shown as examples.

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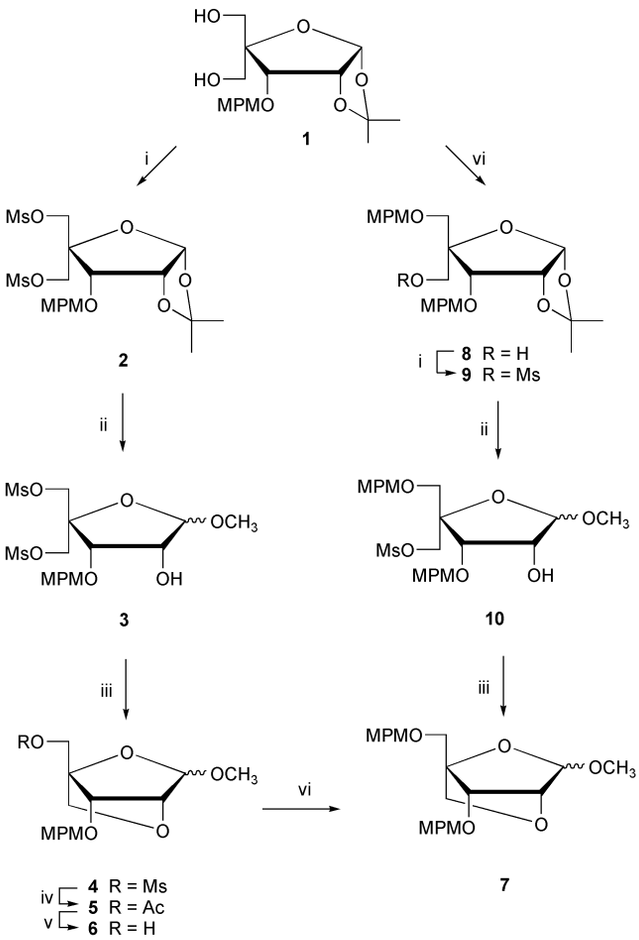
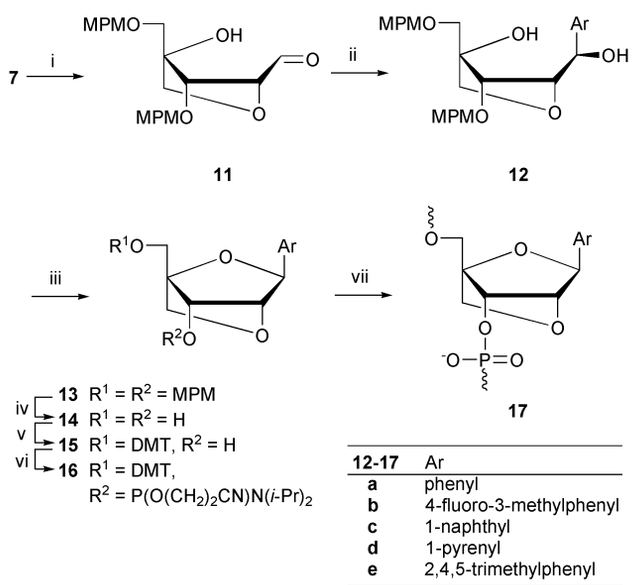


Scheme 1 *Reagents and conditions (and yields)*: i) MsCl, pyridine (synthesis of **2**: 93%; synthesis of **9**: 89%); ii) H_2O–HCl–CH_3OH (1 : 1.5 : 8.5) (synthesis of **3**: 86%; synthesis of **10**: 74%); iii) NaH, DMF [synthesis of **4**: major isomer (74%) + minor isomer (13%); synthesis of **7** from **10**: obtained as a mixture of **7** and **11**]; iv) KOAc, dioxane, 18-crown-6 (isomer obtained from the major anomer of **4**: 91%; isomer obtained from the minor anomer of **4**: 85%); v) saturated methanolic ammonia (isomer obtained from the major anomer of **4**: 88%; isomer obtained from the minor anomer of **4**: 92%); vi) *p*-methoxybenzyl chloride, NaH, THF (isomer obtained from the major anomer of **4**: 80%; isomer obtained from the minor anomer of **4**: 69%). MPM = *p*-methoxybenzyl.

Results and discussion

Synthesis of phosphoramidites 16a–16e and oligomers ON2–ON6 and ON8–ON10 containing monomers (17a–17e)

It was decided to synthesize oligomers **ON2–ON6** and **ON8–ON10** containing the derivatives **17a**–**17e** (Fig. 1, Table 1, Scheme 2) all based on the LNA-type 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl moiety which is known to adopt a locked C3′-*endo* RNA-like furanose conformation.[10,11,14,19] The syntheses of the phosphoramidite building blocks **16a**–**16e** suitable for incorporation of the LNA-type aryl *C*-glycosides **17a**–**17e** are shown in Scheme 1 and Scheme 2. In the design of an appropriate synthetic route, it was decided to utilize a strategy similar to the one described for structurally closely related compounds but with heterocyclic aglycons.[16–18] Thus, stereoselective attack of Grignard reagents of various heterocycles on a carbonyl group of an aldehyde corresponding to aldehyde **11** (Scheme 2) (but with two *O*-benzyl groups instead of the two *p*-methoxybenzyl groups of aldehyde **11**) has been reported *en route* to conformationally locked *C*-nucleosides.[16–18] The key intermediate in the synthetic route selected herein, namely the novel aldehyde **11**, was synthesized from the known furanoside **1**[20] following two different routes. In general, *p*-methoxybenzyl protection was preferred instead of benzyl protection as removal of the benzyl protection at a later stage (*i.e.*, during conversion of **13** to **14**) could also likely result in the cleavage of the benzylic O4′–C1′ bond present, *e.g.*, in compound **14** (Scheme 2). In one route to obtain aldehyde **11**, regioselective *p*-methoxybenzylation of the furanoside **1**, followed by mesylation and methanolysis yielded the anomeric mixture of the methyl furanosides **10**. Base-induced cyclization followed by acetal hydrolysis afforded the aldehyde **11** in 24% overall yield from **1** (Scheme 1 and Scheme 2). This yield was improved to 39% following a different strategy. Thus, di-*O*-mesylation of **1** followed by methanolysis and base-induced intramolecular nucleophilic attack from the 2-OH group afforded the cyclized anomeric mixture of methyl furanoside **4**. Substitution of the remaining mesyloxy group of **4** with an acetate group, followed by deacetylation, *p*-methoxybenzylation and acetal hydrolysis afforded the required aldehyde **11** (Scheme 1 and Scheme 2). The intermediate **7**, obtained by both synthetic routes, was found to be quite unstable under acidic conditions, and on silica gel column it underwent hydrolysis to give the aldehyde **11**. However, an analytical sample of **7** was obtained by rapid fractionation on a silica gel column (1% Et_3N was added to the eluent used).



12-17	Ar
a	phenyl
b	4-fluoro-3-methylphenyl
c	1-naphthyl
d	1-pyrenyl
e	2,4,5-trimethylphenyl

Scheme 2 *Reagents and conditions (and yields)*: i) 80% AcOH (82%); ii) ArMgBr, THF (**12a**: 88%; **12b**: 85%, **12c**: 95%) **12d**: 89%; **12e**: 88%); iii) TMAD, Bu_3P, C_6H_6 (**13a**: 77%; **13b**: 84%; **13c**: 78%; **13d**: 79%; **13e**: 80%); iv) DDQ, CH_2Cl_2, H_2O (**14a**: 66%; **14b**: 67%; **14c**: 67%; **14d**: 75%; **14e**: 65%); v) dimethoxytrityl chloride (DMTCl), pyridine (**15a**: 71%; **15b**: 61%; **15c**: 60%; **15d**: 61%; **15e**: 78%); vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (**16a**: 66%; **16b**: 66%; **16c**: 60%; **16d**: 68%; **16e**: 63%); vii) DNA synthesizer.

Coupling of the aldehyde **11** with different aryl Grignard reagents yielded stereoselectively one epimer of each of the compounds **12a**–**12e** in good yields (see Experimental section and the captions of Scheme 1 and Scheme 2 for further details on this and other synthetic steps). Hari *et al.* have used a similar synthetic strategy to obtain structurally closely related *C*-glycosides with heterocyclic aglycons.[18] The chelation model presented[18] as a possible explanation for the obtained stereoselectivities could also apply for the Grignard reactions to give **12a**–**12e**. Each of the diols **12a**–**12e** was cyclized under Mitsunobu conditions (*N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD), PBu_3) to afford the bicyclic β-configured *C*-nucleoside derivatives **13a**–**13e**. Oxidative removal of the *p*-methoxybenzyl protection groups was achieved in satisfactory yields using DDQ. Subsequently, selective 4,4′-dimethoxytritylation (to give compounds **15a**–**15e**) followed by phosphitylation afforded the phosphoramidite building blocks **16a**–**16e** in satisfactory yields. The configurations of compounds **13**,

and thus also compounds **11**, **12** and **14**–**17**, were assigned based on ^{1}H NMR spectroscopy, including NOE experiments. Thus, selective irradiation of the H3′ proton of compounds **13a**–**13e** gave enhancement of signals of the aromatic aglycon (3.0% for **13a**, 2.7% for **13b**, 6.2% for **13c**, 7.0% for **13d** and 6.8% for **13e**) which confirms the *cis*-positioning of the H3′-proton and the aglycon on the furanose ring and furthermore supports an *N*-type furanose conformation. In addition, a single-crystal X-ray diffraction study was performed of the phenyl analogue **14a**. The molecules in the structure are connected through hydrogen bonds, especially O–H ⋯ O interactions, thus forming infinite chains extending along the crystallographic *b*-direction. The obtained structure shown in Fig. 2 verifies the constitution and the relative configuration of

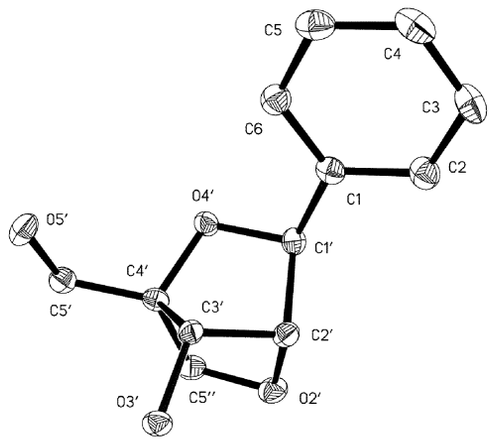


Fig. 2 Molecular structure (ORTEP plot) of the bicyclic *C*-glycoside **14a**.‡

the bicyclic *C*-nucleoside as well as its locked *N*-type furanose conformation. From the measured torsions describing the furanose ring and following the generel definition, the pseudorotational phase angle was calculated to be 20.5°.[21] The absolute configuration follows from the stereochemically pure starting materials used and the applied synthetic route (Schemes 1 and 2).

All oligomers **ON1–ON10** were prepared on a 0.2 μmol scale using the phosphoramidite approach (see the Experimental section for details). The stepwise coupling efficiencies of the phosphoramidites **16a**–**16c** (10 min coupling time) and phosphoramidites **16d** and **16e** (20 min coupling time) were >96% and of unmodified deoxynucleoside phosphoramidites and 2′-*O*-methylribonucleoside phosphoramidites (with standard coupling time) >99%, in all cases using 1*H*-tetrazole as activator. 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 **ON2–ON6** and **ON8–ON10** was verified by MALDI-MS analysis and their purity (>80%) by capillary gel electrophoresis.

Thermal denaturation studies

The hybridization of the oligonucleotides **ON1–ON10** (Table 1) towards four 9-mer DNA targets with the central base being each of four natural bases was studied by thermal denaturation experiments (T_m measurements; see the Experimental section for details). Compared to the DNA reference **ON1**, introduction of one of the LNA-type *C*-glycoside monomers **17a**–**17e** leads to significantly reduced thermal stability of the resulting duplexes. Thus, for the phenyl monomer **17a** (**ON2**) as example, T_m values in the range of 5–12 °C were observed, the most stable duplexes being formed with the target strand containing

‡ CCDC reference number 189923. See http://www.rsc.org/suppdata/p1/b2/b206626b/

Table 1 Thermal denaturation experiments (T_m values shown) for **ON1**–**ON10** 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[11]	5′-d(GTGATATGC)	28	11	12	19
ON2[1]	5′-d(GTGA**17a**ATGC)	12	5	6	7
ON3	5′-d(GTGA**17b**ATGC)	15	7	6	8
ON4	5′-d(GTGA**17c**ATGC)	15	7	6	9
ON5[1]	5′-d(GTGA**17d**ATGC)	18	17	18	19
ON6	5′-d(GTGA**17e**ATGC)	13	6	6	7
ON7[1]	5′-d[2′-OMe(GTGATATGC)]	35	14	19	21
ON8	5′-d[2′-OMe(G**T^L**GA**17b**A**T^L**GC)]	31	25	26	27
ON9	5′-d[2′-OMe(G**T^L**GA**17c**A**T^L**GC)]	34	27	27	32
ON10[1]	5′-d[2′-OMe(G**T^L**GA**17d**A**T^L**GC)]	39	38	37	40

[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 (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 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 monomer; See Fig. 1 for structures of **T^L**, 2′-OMe-RNA monomers and monomers **17a**–**17e**; DNA sequences are shown as d(sequence) and 2′-OMe-RNA sequences as 2′-OMe(sequence).

an adenine base opposite to monomer **17a**. Similar results were obtained for the 4-fluoro-3-methylphenyl (**17b**), 1-naphthyl (**17c**) and 2,4,5-trimethylphenyl (**17e**) derivatives (**ON3**, **ON4** and **ON6**). Thus, these four derivatives stabilize the duplexes compared to the corresponding LNA-type abasic monomer,[22] but due to the preferential binding to the target DNA with the central adenine monomer, universal hybridization is not achieved (Table 1). Universal hybridization has in fact been reported with the corresponding DNA-type monomer containing 2,4,5-trimethylphenyl as aglycon.[23] This DNA-type monomer was originally designed as a hydrophobic isostere of a thymidine residue.[24] As selective pairing with an adenine monomer was not realized for this aglycon as a DNA-type monomer,[23] the preferred pairing of LNA-type monomer **17e** (**ON6**) with an adenine monomer is noteworthy.

The pyrenyl LNA nucleotide **17d** displays more encouraging properties in relation to universal hybridization (**ON5**, Table 1). Thus, compared to **ON2–ON4** and **ON6**, less destabilized hybridization towards all four complements is obtained with **ON5**. Furthermore, universal hybridization is obtained as shown by the four T_m values all being within 17–19 °C. With respect to universal hybridization, the LNA-type pyrenyl monomer **17d** parallels the corresponding pyrene DNA derivative[5] which, however, relative to the fully matched DNA–DNA duplex, showed less pronounced destabilization than **17d** when incorporated into a DNA strand [$\Delta T_m \approx -10$ °C for **17d** (**ON5** relative to **ON1**) and $\Delta T_m \approx -5$ °C for a pyrenyl DNA monomer (incorporated into a 12-mer DNA sequence[5])]. It therefore appears that stacking (or intercalation) by the pyrene moiety is disfavoured by conformationally locking the furanose ring into an *N*-type (^3E) conformation. However, comparison of the thermal stabilities of **ON2**, **ON3**, **ON4** and **ON6** with **ON5** strongly indicates some productive interaction of the pyrenyl moiety with the helix, *e.g.* intercalation.

In order to study the effect of the LNA-type β-*C*-aryl nucleotide monomers in A-type duplexes we synthesized **ON7–ON10** (Table 1). **ON7** was selected as the reference strand which, being composed entirely of 2′-OMe-RNA monomers, is known to structurally mimic an RNA strand.[25] As mentioned above, increased binding affinity of universal hybridization probes is considered important and we therefore constructed **ON8–ON10** as a mixture of six 2′-OMe-RNA monomers, one central LNA-type β-*C*-aryl glycoside monomer (**17b**, **17c** or **17d**), and two affinity-enhancing LNA thymine monomers **T^L**. With

respect to hybridization selectivity, a pattern similar to the one described above for modified DNA oligomers was observed, *i.e.*, preferential hybridization towards the target DNA containing the central adenine monomer for the 4-fluoro-3-methylphenyl (**17b**) and 1-naphthyl (**17c**) monomers, and universal hybridization for the pyrenyl monomer (**17d**) (Table 1; T_m values for **ON10**: 37, 38, 39 and 40 °C towards the four targets). The 2′-OMe-RNA reference **ON7** binds to the DNA complement with slightly increased thermal stability compared to the DNA reference **ON1** while still obeying the Watson–Crick base-pairing rules. The affinity-enhancing effect of the LNA thymine monomer $\mathbf{T^L}$ is reflected in the satisfactory thermal stabilites obtained for **ON8–ON10**. Especially noteworthy is the fact that **ON10** containing the pyrenyl monomer **17d** displays higher T_m values than the two fully complementary reference duplexes **ON1**:DNA (T_m = 28 °C) and **ON7**:DNA (T_m = 35 °C). These data demonstrate that the pyrene LNA monomer **17d** displays universal hybridization behaviour both in a DNA context (**ON5**) and in an RNA-like context (**ON10**), in the latter case with satisfactory binding affinities. These results clearly indicated that the problem of decreased affinity of the known universal hybridization probes can be solved by the incorporation of high-affinity monomers, *e.g.* 2′-OMe-RNA and/or LNA monomers. Furthermore it should be noted that we have earlier in a systematic thermal denaturation study demonstrated satisfactory discriminatory ability of the two monomers neighbouring monomer **17d** in **ON10**,[1] contrary to what has been reported for a DNA strand containing the universal 3-nitropyrrole DNA nucleotide.[26] The properties of other classes of conformationally restricted aryl-*C*-nucleotides remain to be studied, as does the ability of *e.g.* the pyrenyl monomer **17d** to selectively stabilize duplexes when incorporated opposite to an abasic site as has been reported for the corresponding DNA-type monomer.[5] Furthermore, the results obtained call for studies of LNA-type monomers containing other hydrophobic aglycons so far exclusively published as DNA-type monomers.[6,23,27–29]

Conclusion

Synthesis of a series of LNA-type β-configured *C*-aryl nucleosides has been accomplished as has their efficient incorporation into short DNA and 2′-OMe-RNA/LNA strands using the phosphoramidite approach. It has been shown that universal hybridization is achievable with a conformationally restricted monomer as demonstrated for the pyrene LNA monomer **17d**, both in a DNA and in an RNA context. Importantly, increase of the binding affinity of oligonucleotide probes for universal hybridization can be obtained by the introduction of affinity-enhancing monomers.

Experimental section §

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 plates with flourescense indicator (SiO_2-60, F-254) visualizing 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 flash column chromatography. Petroleum ether of the distillation range 60–80 °C was used. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure and dried overnight 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 and follow the standard carbohydrate/nucleoside nomenclature. Coupling constants (*J* values) are given in hertz. The assignments of methylene protons, when given, may be interchanged. Bicyclic compounds are named according to the von Baeyer nomenclature, whereas the atom numbering in the assignments follows the standard carbohydrate/nucleoside nomenclature. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a Kratos MS50TC spectometer 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.

1,2-*O*-Isopropylidene-5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-α-D-*erythro*-pentofuranose (2)

Methanesulfonyl chloride (8.6 g, 7.5 mmol) was added dropwise to a stirred solution of 4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-3-*O*-*p*-methoxybenzyl-α-D-*erythro*-pentofuranose[20] (**1**) (10.0 g, 29.4 mmol) in anhydrous pyridine (30 cm^3) and the reaction mixture was stirred overnight at rt. The mixture was evaporated to dryness under reduced pressure to give a residue which was co-evaporated with toluene (2 × 25 cm^3), dissolved in CH_2Cl_2 (200 cm^3) and washed successively with saturated aq. $NaHCO_3$ (2 × 100 cm^3) and brine (50 cm^3). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The colourless viscous oil obtained was purified by column chromatography [0.5–1% (v/v) MeOH in CH_2Cl_2 as eluent], followed by crystallization from MeOH, to give furanose **2** as a white solid material (13.6 g, 93%). R_f 0.57 (CH_2Cl_2/MeOH 95:5, v/v); δ_H ($CDCl_3$) 7.30 (2H, d, *J* 8.7), 6.90 (2H, d, *J* 8.5), 5.78 (1H, d, *J* 3.7), 4.86 (1H, d, *J* 12.0), 4.70 (1H, d, *J* 11.4), 4.62 (1H, dd, *J* 5.0 and 3.8), 4.50 (1H, d, *J* 11.1), 4.39 (1H, d, *J* 12.3), 4.31 (1H, d, *J* 11.0), 4.17 (1H, d, *J* 5.1), 4.11 (1H, d, *J* 11.0), 3.81 (3H, s), 3.07 (3H, s), 2.99 (3H, s), 1.68 (3H, s), 1.34 (3H, s); δ_C ($CDCl_3$) 159.8, 129.9, 128.8, 114.1, 114.0, 104.5, 83.2, 78.0, 77.9, 72.6, 69.6, 68.8, 55.4, 38.1, 37.5, 26.3, 25.7.

Methyl 5-*O*-methanesulfonyl-4-*C*-methanesulfonyloxymethyl-3-*O*-(*p*-methoxybenzyl)-α,β-D-*erythro*-pentofuranoside (3)

A suspension of furanoside **2** (13.5 g, 27.2 mmol) in a mixture of H_2O (45 cm^3) and 15% HCl in MeOH (450 cm^3, w/w) was stirred at rt for 72 h. The mixture was carefully neutralized by addition of saturated aq. $NaHCO_3$ (100 cm^3) followed by addition of $NaHCO_3$ (s), whereupon the mixture was evaporated to dryness under reduced pressure. H_2O (100 cm^3) was added, 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 then evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2 × 25 cm^3) and purified by column chromatography [1–2% (v/v) MeOH in CH_2Cl_2] to give furanoside **3** as an anomeric mixture (clear oil, 11.0 g, 86%, ratio between anomers ~6 : 1). R_f 0.39, 0.33 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$, major anomer) 7.28 (2H, d, *J* 8.0), 6.91 (2H, d, *J* 8.7), 4.87 (1H, s), 4.62 (1H, d, *J* 11.2), 4.53 (1H, d, *J* 11.4), 4.41 (2H, s), 4.31 (1H, d, *J* 9.7), 4.25 (1H, d, *J* 4.7), 4.06 (1H, d, *J* 9.5), 3.99 (1H, br s),

§ Copies of the ^{13}C NMR spectra of compounds **2**, **3**, **4**–**7** (both isomers), **9**–**11**, **12a**–**12e**, **13a**–**13e**, **14a**–**14e** and **15a**–**15e** and copies of the ^{31}P NMR spectra of compounds **16a**–**16e** were enclosed with the manuscript on submission.

3.81 (3H, s), 3.33 (3H, s), 3.06 (3H, s), 3.03 (3H,s); δ_C ($CDCl_3$, major anomer) 160.0, 130.1, 128.5, 114.3, 107.8, 81.7, 81.2, 73.9, 73.6, 69.7, 69.6, 55.5, 55.4, 37.5, 37.4.

(1*R*,3*RS*,4*R*,7*S*)-1-Methylsulfonyloxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (4)

To a stirred solution of the anomeric mixture **3** (10.9 g, 23.2 mmol) in anhydrous DMF (50 cm^3) at 0 °C was during 10 min added sodium hydride (2.28 g of a 60% suspension in mineral oil (w/w), 95.2 mmol) and the mixture was stirred for 12 h at rt. Ice-cold H_2O (200 cm^3) was slowly added and extraction was performed using EtOAc (3 × 200 cm^3). The combined organic phase was washed successively with saturated aq. $NaHCO_3$ (2 × 100 cm^3) and brine (50 cm^3), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [0.5–1% (v/v) MeOH in CH_2Cl_2] to give first the major isomer (6.42 g, 74%) and then [1.5% (v/v) MeOH in CH_2Cl_2] the minor isomer (1.13 g, 13%), both as clear oils. R_f 0.56, 0.45 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$, major isomer) 7.26 (2H, d, *J* 8.5), 6.89 (2H, d, *J* 8.1), 4.80 (1H, s), 4.61–4.44 (4H, m), 4.10–4.09 (2H, m), 3.99 (1H, d, *J* 7.5), 3.81 (3H, s), 3.69 (1H, d, *J* 7.4), 3.36 (3H, s), 3.05 (3H, s); δ_C ($CDCl_3$, major isomer) 159.6, 129.5,129.3, 114.0, 105.3, 83.2, 78.6, 77.2, 72.1, 71.8, 66.3, 55.6, 55.4, 37.8; δ_H ($CDCl_3$, minor isomer) 7.27 (2H, d, *J* 8.5), 6.89 (2H, d, *J* 8.7), 4.99 (1H, s), 4.63–4.39 (4H, m), 4.18 (1H, s), 4.04 (1H, d, *J* 8.7), 3.94–3.91 (2H, m), 3.81 (3H, s), 3.47 (3H, s), 3.05 (3H, s); δ_C ($CDCl_3$, minor isomer) 159.7, 129.6, 129.0, 114.1, 104.4, 86.4, 79.4, 77.1, 72.3, 71.9, 66.2, 56.4, 55.4, 37.7.

(1*R*,3*RS*,4*R*,7*S*)-1-Acetoxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (5)

To a stirred solution of furanoside **4** (major isomer, 6.36 g, 17.0 mmol) in anhydrous dioxane (25 cm^3) was added 18-crown-6 (9.0 g, 34.1 mmol) and KOAc (8.4 g, 85.6 mmol). The stirred mixture was heated under reflux for 12 h and subsequently evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 cm^3) and washing was performed, successively, with saturated 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 [1% (v/v) MeOH in CH_2Cl_2] to give furanoside **5** (one isomer) as a colourless oil (5.23 g, 91%). R_f 0.47 (EtOAc–petroleum ether 75:25, v/v); δ_H ($CDCl_3$) 7.26 (2H, d, *J* 8.2), 6.89 (2H, d, *J* 8.9), 4.80 (1H, s), 4.59 (1H, d, *J* 11.5), 4.49 (1H, d, *J* 11.6), 4.47 (1H, d, *J* 12.4), 4.27 (1H, d, *J* 12.7), 4.09 (1H, s), 4.05 (1H, s), 3.99 (1H, d, *J* 7.4), 3.80 (3H, s), 3.71 (1H, d, *J* 7.5), 3.37 (3H, s), 2.06 (3H, s); δ_C ($CDCl_3$) 170.7, 159.5, 129.6, 129.4, 113.9, 105.1, 83.3, 78.9, 77.2, 72.1, 72.0, 61.1, 55.5, 55.3, 20.8. Similarly, furanoside **4** (minor isomer, 450 mg, 1.2 mmol) was refluxed with KOAc (600 mg, 6.1 mmol) and 18-crown-6 (600 mg, 2.3 mmol) in dioxane (5 cm^3) for 12 h. After work-up as described for the reaction with the major isomer of **4**, the crude product obtained was purified by column chromatography [50–60% (v/v) EtOAc in petroleum ether] to yield furanoside **5** (one isomer) as a colourless oil (345 mg, 85%). R_f 0.33 (EtOAc–petroleum ether 75:25, v/v); δ_H ($CDCl_3$) 7.26 (2H, d, *J* 8.4), 6.89 (2H, d, *J* 8.7), 4.98 (1H, s), 4.61 (1H, d, *J* 11.5), 4.49 (1H, d, *J* 11.8), 4.35 (1H, d, *J* 12.4), 4.28 (1H, d, *J* 12.7), 4.18 (1H, s), 4.02 (1H, d, *J* 7.5), 3.92 (1H, d, *J* 8.1), 3.87 (1H, s), 3.81 (3H, s), 3.48 (3H, s), 2.06 (3H, s); δ_C ($CDCl_3$) 170.6, 159.6, 129.4, 129.3, 114.0, 104.3, 86.6, 79.5, 77.1, 72.6, 71.7, 60.8, 56.4, 55.3, 20.8.

(1*S*,3*RS*,4*R*,7*S*)-1-Hydroxymethyl-3-methoxy-7-(*p*-methoxybenzyloxy)-2,5-dioxabicyclo[2.2.1]heptane (6)

A solution of furanoside **5** (isomer obtained from the major anomer of **4**, 5.16 g, 15.3 mmol) in saturated methanolic ammonia (200 cm^3) was stirred at room temperature for 48 h. The reaction mixture was evaporated to dryness under reduced pressure, coevaporated with toluene (2 × 50 cm^3), and the residue purified by column chromatography [60–70% (v/v) EtOAc in petroleum ether] to give furanoside **6** (one isomer) as a white solid material (3.98 g, 88%). R_f 0.31 (EtOAc–petroleum ether 75:25, v/v); δ_H ($CDCl_3$) 7.27 (2H, d, *J* 8.4), 6.88 (2H, d, *J* 8.5), 4.80 (1H, s), 4.59 (1H, d, *J* 11.2), 4.52 (1H, d, *J* 11.6), 4.09 (2H, s), 3.97 (1H, d, *J* 7.4), 3.85 (2H, br s), 3.80 (3H, s), 3.64 (1H, d, *J* 7.4), 3.37 (3H, s), 2.11 (1H, br s); δ_C ($CDCl_3$) 159.5, 129.8, 129.4, 113.9, 105.2, 85.6, 78.3, 77.4, 72.0, 71.8, 58.8, 55.5, 55.3. Similarly, furanoside **5** (isomer obtained from the minor anomer of **4**, 300 mg, 0.89 mmol) was deacylated with saturated methanolic ammonia (15 cm^3). The crude product obtained after the work-up procedure was purified by column chromatography [60–70% (v/v) EtOAc in petroleum ether] to give furanoside **6** (one isomer) as a white solid material (242 mg, 92%). R_f 0.16 (EtOAc–petroleum ether 75:25, v/v); δ_H ($CDCl_3$) 7.28 (2H, d, *J* 8.7), 6.88 (2H, d, *J* 8.8), 4.97 (1H, s), 4.61 (1H, d, *J* 11.5), 4.54 (1H, d, *J* 11.6), 4.16 (1H, s), 3.99 (1H, d, *J* 7.8), 3.94 (1H, s), 3.86 (1H, d, *J* 7.9), 3.82 (2H, br s), 3.80 (3H, s), 3.47 (3H, s), 2.11 (1H, m); δ_C ($CDCl_3$) 159.5, 129.5, 129.4, 114.0, 104.3, 89.1, 78.9, 77.4, 72.3, 71.8, 58.7, 56.3, 55.4.

(1*S*,3*SR*,4*R*,7*S*)-3-Methoxy-7-(*p*-me thoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-2,5-dioxabicyclo[2.2.1] heptane (7)

Sodium hydride (50 mg, 60% in mineral oil, 12.4 mmol) was added to anhydrous THF (3 cm^3) under N_2 and the suspension was cooled to 0 °C. A solution of furanoside **6** (isomer obtained from the major anomer of **4**, 220 mg, 0.74 mmol) in anhydrous THF (1 cm^3) was added dropwise. The temperature of the mixture was allowed to increase to rt and stirring at rt was continued for 15 min. *p*-Methoxybenzyl chloride (188 mg, 1.2 mmol) was added dropwise and the resulting mixture stirred for 48 h at rt. Additional *p*-methoxybenzyl chloride (188 mg, 1.20 mmol) was added and the resulting mixture stirred for another 24 h. The mixture was cooled to 0 °C and ice-cold water (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, evaporated to dryness under reduced pressure. The residue was purified by rapid column chromatography [20–25% EtOAc in petroleum ether containing 1% Et_3N, v/v/v] to give furanoside **7** (one isomer) as a colourless oil (247 mg, 80%). R_f 0.62 (EtOAc–petroleum ether 75:25, v/v); δ_H ($CDCl_3$) 7.27–7.20 (4H, m), 6.90–6.84 (4H, m), 4.79 (1H, s), 4.61–4.52 (3H, m), 4.47 (1H, d, *J* 11.3), 4.07 (1H, s), 4.04 (1H, s), 3.95 (1H, d, *J* 7.8), 3.83–3.79 (7H, m), 3.76–3.72 (2H, m), 3.38 (3H, s); δ_C ($CDCl_3$) 159.4, 159.3, 130.1, 129.8, 129.4, 129.3, 128.7, 114.0, 113.9, 113.8, 105.0, 85.2, 79.0, 77.3, 73.4, 72.4, 71.9, 66.3, 55.5, 55.3. Similarly, furanoside **6** (isomer obtained from the minor anomer of **4**, 202 mg, 0.68 mmol) was reacted with *p*-methoxybenzyl chloride (391 mg, 2.5 mmol) in the presence of NaH (45 mg,11.2 mmol) and anhydrous THF (4 cm^3). The crude product obtained after the work-up procedure was purified by rapid column chromatography [35–40% EtOAc in petroleum ether containing 1% Et_3N, v/v/v] to give furanoside **7** (one isomer) as a colourless oil (196 mg, 69%). R_f 0.33 (EtOAc–petroleum ether 75 : 25, v/v); δ_H ($CDCl_3$) 7.25 (2H, d, *J* 8.5), 7.21 (2H, d, *J* 8.6), 6.86 (2H, d, *J* 8.3), 6.85 (2H, d, *J* 8.5), 4.99 (1H, s), 4.58 (1H, d, *J* 11.6), 4.55 (1H, d, *J* 11.5), 4.49 (1H, d, *J* 12.0), 4.48 (1H, d, *J* 11.7), 4.14 (1H, s), 3.94 (1H, d, *J* 7.8), 3.94 (1H, s), 3.89 (1H, d, *J* 7.8), 3.80 (3H, s), 3.79 (3H, s), 3.65 (2H, br s), 3.49 (3H, s); δ_C ($CDCl_3$) 159.4, 129.9, 129.6, 129.5, 129.4, 113.9, 113.8, 104.1, 88.5, 79.3, 77.3, 73.4, 72.7, 71.7, 65.4, 56.4, 56.3, 55.3.

4-*C*-Methanesulfonyloxymethyl-3,5-di-*O*-(*p*-methoxybenzyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (9)

3,5-Di-*O*-(*p*-methoxybenzyl)-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-ribofuranose[20] (**8**) (3.2 g, 6.95 mmol) was mesylated using MsCl (2.00 g, 17.5 mmol) and pyridine (10 cm^3) following the same procedure as described for the synthesis of compound **2**. After work-up, the colourless viscous oil was purified by column chromatography [1% (v/v) MeOH in CH_2Cl_2] to give derivative **9** as a clear oil (3.17 g, 89%). R_f 0.45 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.22 (2H, d, *J* 8.9), 7.18 (2H, d, *J* 8.7), 6.86 (4H, d, *J* 8.3), 5.76 (1H, d, *J* 3.8), 4.83 (1H, d, *J* 12.0), 4.64 (1H, d, *J* 11.6), 4.59 (1H, m), 4.49–4.35 (4H, m), 4.24 (1H, d, *J* 5.3), 3.80 (6H, s), 3.56 (1H, d, *J* 10.5), 3.45 (1H, d, *J* 10.5), 3.06 (3H, s), 1.67 (3H, s), 1.33 (3H, s); δ_C ($CDCl_3$) 159.6, 159.4, 129.9, 129.8, 129.7, 129.5, 129.4, 129.3, 114.0, 113.9, 113.8, 113.7, 113.6, 104.5, 84.9, 78.6, 78.1, 73.4, 72.4, 71.0, 69.9, 55.3, 38.0, 26.4, 25.9.

Methyl 4-*C*-methanesulfonyloxymethyl-3,5-di-*O*-(*p*-methoxybenzyl)-α,β-D-ribofuranose (10)

Methanolysis of furanoside **9** (3.1 g, 5.76 mmol) was performed using a mixture of a solution of 15% HCl in MeOH (w/w, 120 cm^3) and H_2O (12 cm^3) following the procedure described for the synthesis of compound **3**. After work-up, the crude product was purified by column chromatography [eluting with 0.5–1% (v/v) MeOH in CH_2Cl_2] to give the major anomer of **10** (1.71 g, 58%) and [eluting with 1–1.5% (v/v) MeOH in CH_2Cl_2] the minor anomer of **10** (0.47 g, 16%), both as clear oils. R_f 0.31, 0.24 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_C (major anomer, $CDCl_3$) 159.7, 159.4, 129.9, 129.8, 129.7, 129.5, 129.1, 128.7, 114.0, 113.9, 107.5, 83.2, 81.6, 74.1, 73.4, 73.1, 72.3, 70.7, 65.0, 55.4, 55.0, 37.3.

Alternative preparation of furanoside 7

Ring closure of furanoside **10** (major anomer, 1.68 g, 3.28 mmol) was achieved using NaH (60% suspension in mineral oil (w/w), 0.32 g, 13.1 mmol) in anhydrous DMF (10 cm^3) following the procedure described for the synthesis of compound **4** to give a crude product tentatively assigned as a mixture of furanoside **7** and aldehyde **11** (see below) (1.13 g).

(2*R*,3*S*,4*S*)-4-Hydroxy-3-(*p*-methoxybenzyloxy)-4-(*p*-methoxybenzyloxymethyl)tetrahydrofuran-2-carbaldehyde (11)

A solution of furanoside **7** (isomer obtained from the major anomer of **4**, 217 mg, 0.521 mmol) in 80% aqueous acetic acid (w/w, 5 cm^3) was stirred at 50 °C for 4 h. The mixture was evaporated to dryness under reduced pressure and the residue was successively coevaporated with absolute ethanol (3 × 5 cm^3) and toluene (3 × 5 cm^3) and purified by column chromatography [40–45% (v/v) EtOAc in petroleum ether] to give aldehyde **11** as a colourless oil (172 mg, 82%). R_f 0.37 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 9.64 (1H, br s), 7.25–7.17 (4H, m), 6.87–6.84 (4H, m), 4.59 (1H, d, *J* 11.7), 4.51–4.41 (2H, m), 4.35 (1H, s), 3.92–3.86 (2H, m), 3.79 (6H, s), 3.77–3.73 (3H, m), 3.45 (1H, d, *J* 9.1); δ_C ($CDCl_3$) 203.6, 159.5, 159.4, 129.7, 129.6, 129.5, 129.2, 114.0, 113.9, 113.8, 87.3, 86.8, 81.0, 75.1, 73.5, 71.7, 67.6, 55.3.

Alternative preparation of aldehyde 11

A solution of crude furanoside **7** (as a mixture with **11** prepared as described above in the alternative preparation of furanoside **7** (5.80 g) in 80% glacial acetic acid (100 cm^3) was stirred at 50 °C for 4 h. The solvent was distilled off under reduced pressure and the residue was successively coevaporated with absolute ethanol (3 × 25 cm^3) and toluene (2 × 25 cm^3) and purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] to give aldehyde **11** as a colourless oil (4.60 g). Analytical data as listed above.

General procedure for the reaction of arylmagnesium bromides with aldehyde 11 to give compounds 12a–12e

A solution of aldehyde **11** in anhydrous THF (10 cm^3) was added dropwise during 5 min to a stirred solution of the arylmagnesium bromide dissolved in anhydrous THF at 0 °C. The temperature was allowed to rise to rt and the mixture was stirred for 12 h. The mixture was evaporated to dryness under reduced pressure and the residue diluted with CH_2Cl_2 and washed several times with saturated aq. NH_4Cl. The organic phase was dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. Column chromatography of the crude product thus obtained afforded compound **12a**–**12e** (for **12a**–**12d** a minor impurity which could be the diastereoisomeric addition product was detected in the NMR spectra; this impurity could neither be isolated nor characterized).

(2*S*,3*S*,4*S*)-4-Hydroxy-2-[(*R*)-hydroxy(phenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (12a). Grignard reaction between phenylmagnesium bromide (1.0 M solution in THF, 14.2 cm^3, 14.2 mmol) and aldehyde **11** (515 mg, 1.28 mmol) afforded tetrahydrofuran **12a**. The crude product was purified by column chromatography [4% (v/v) MeOH in CH_2Cl_2] to give tetrahydrofuran **12a** (540 mg, 88%) as a colourless oil. R_f 0.34 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 7.40–7.19 (7H, m), 6.91–6.73 (6H, m), 4.73 (1H, d, *J* 6.4), 4.48 (2H, s), 4.08 (2H, s), 3.88 (1H, d, *J* 9.5), 3.79 (1H, m), 3.78 (3H, s), 3.76 (3H, s), 3.75–3.69 (2H, m), 3.50 (1H, d, *J* 9.4), 3.45 (1H, s), 3.42 (1H, br s), 3.26 (1H, br s); δ_C ($CDCl_3$) 159.5, 159.3, 140.7, 129.7, 129.6, 129.5, 129.2, 128.5, 128.0, 127.3, 113.9, 113.8, 113.7, 89.4, 84.6, 81.8, 75.3, 74.7, 73.5, 71.6, 69.3, 55.3; MALDI-HRMS: *m*/*z* 503.2019 ([M + Na]$^+$, $C_{28}H_{32}O_7Na^+$ calc. 503.2040).

(2*S*,3*S*,4*S*)-4-Hydroxy-2-[(*R*)-hydroxy(4-fluoro-3-methylphenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (12b). Grignard reaction between 4-fluoro-3-methylphenylmagnesium bromide (1.0 M solution in THF, 15.0 cm^3, 15.0 mmol) and aldehyde **11** (603 mg, 1.5 mmol) afforded tetrahydrofuran **12b**. The crude product was purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] to give tetrahydrofuran **12b** (611 mg, 85%) as a colourless oil. R_f 0.34 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 7.24–7.12 (5H, m), 6.98–6.84 (5H, m), 6.77 (1H, d, *J* 8.5), 4.65 (1H, dd, *J* 2.8 and 6.4), 4.49 (2H, s), 4.15 (2H, s), 4.01 (1H, dd, *J* 2.3 and 6.5), 3.87 (1H, d, *J* 9.3), 3.79 (3H, s), 3.78 (3H, s), 3.76–3.68 (2H, m), 3.52 (1H, s), 3.47 (1H, d, *J* 10.3), 3.42 (1H, d, *J* 2.9), 3.22 (1H, s), 2.24 (3H, d, *J* 0.8); δ_C ($CDCl_3$) 161.0 (d, *J* 244.4), 159.5, 159.4, 136.2, 136.1, 130.3, 130.2, 129.7, 129.6, 129.5, 129.4, 129.1, 126.1, 126.0, 115.1, 114.8, 114.0, 113.9, 113.8, 113.7, 89.3, 84.5, 81.8, 75.3, 74.0, 73.5, 71.7, 69.2, 55.4, 55.3, 14.7 (d, *J* 3.9); MALDI-HRMS: *m*/*z* 535.2087 ([M + Na]$^+$, $C_{29}H_{33}O_7FNa^+$ calc. 535.2102).

(2*S*,3*S*,4*S*)-4-Hydroxy-2-[(*R*)-hydroxy(1-naphthyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (12c). 1-Bromonaphthalene (1.55 g, 7.5 mmol) was added to a stirred mixture of magnesium turnings (182 mg, 7.5 mmol) and iodine (10 mg) in THF (10 cm^3). The mixture was stirred at 40 °C for 1 h whereupon it was allowed to cool to rt. A solution of aldehyde **11** (603 mg, 1.5 mmol) in THF (10 cm^3) was added slowly and the reaction was stirred for 12 h. The crude product was purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] to give tetrahydrofuran **12c** (756 mg, 95%) as a colourless oil. R_f 0.35 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 8.08 (1H, m), 7.86 (1H, m), 7.79 (1H, d, *J* 8.2), 7.72 (1H, d, *J* 7.2), 7.49–7.44 (3H, m), 7.18 (2H, d, *J* 8.4), 6.84 (2H, d, *J* 8.6), 6.74 (2H, d, *J* 8.7), 6.68 (2H, d, *J* 8.8), 5.52 (1H, dd, *J* 3.7 and 5.6), 4.45 (2H, s), 4.34 (1H, dd, *J* 2.5 and 5.9), 4.03 (1H, d, *J* 11.0), 3.96 (1H, d, *J* 11.0), 3.93 (1H, d, *J* 9.5), 3.80

(1H, d, *J* 9.3), 3.77 (3H, s), 3.75 (1H, d, *J* 2.6), 3.72 (3H, s), 3.68 (1H, d, *J* 9.3), 3.56 (1H, d, *J* 3.7), 3.49 (1H, d, *J* 9.3), 3.34 (1H, s); δ_C ($CDCl_3$) 159.5, 159.3, 136.3, 134.0, 131.0, 129.7, 129.6, 129.5, 129.4, 129.0, 128.6, 126.2, 125.6, 125.5, 123.5, 114.0, 113.8, 113.7, 88.7, 84.7, 81.9, 75.5, 73.5, 71.7, 71.3, 69.3, 55.4, 55.3; MALDI-HRMS: *m*/*z* 553.2199 ($[M + Na]^+$, $C_{32}H_{34}O_7Na^+$ calc. 553.2197).

(2*S*,3*S*,4*S*)-4-Hydroxy-2-[(*R*)-hydroxy(pyren-1-yl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (12d). Tetrahydrofuran **12d** was synthesized from aldehyde **11** (515 mg, 1.28 mmol), 1-bromopyrene (1.0 g, 3.56 mmol), magnesium turnings (155 mg, 6.4 mmol), iodine (10 mg) and THF (20 cm^3) following the procedure described for synthesis of compound **12c** and the general procedure described for the synthesis of compounds **12a**–**12e**. The crude product was purified by column chromatography [3–4% (v/v) MeOH in CH_2Cl_2] to give tetrahydrofuran **12d** (690 mg, 89%) as a pale yellow solid. R_f 0.35 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 8.23 (2H, d, *J* 8.4 and 9.2), 8.19–8.13 (3H, m), 8.05–7.99 (4H, m), 7.14 (2H, d, *J* 8.8), 6.82 (2H, d, *J* 9.0), 6.30 (2H, d, *J* 8.7), 6.20 (2H, d, *J* 8.6), 5.87 (1H, d, *J* 7.2), 4.43 (2H, s), 4.41 (1H, m), 4.01 (1H, d, *J* 9.4), 3.91 (1H, d, *J* 11.8), 3.86 (1H, d, *J* 9.2), 3.77 (1H, d, *J* 1.9), 3.76 (3H, s), 3.70–3.64 (3H, m), 3.52–3.45 (1H, m), 3.44 (3H, s); δ_C ($CDCl_3$) 159.5, 158.9, 133.9, 131.4, 131.1, 130.7, 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, 113.3, 89.5, 83.5, 82.0, 75.7, 73.4, 71.3, 71.0, 69.3, 55.3, 55.0; MALDI-HRMS: *m*/*z* 627.2376 ($[M + Na]^+$, $C_{38}H_{36}O_7Na^+$ calc. 627.2353).

(2*S*,3*S*,4*S*)-4-Hydroxy-2-[(*R*)-hydroxy(2,4,5-trimethylphenyl)methyl]-4-(*p*-methoxybenzyloxy)-3-(*p*-methoxybenzyloxymethyl)tetrahydrofuran (12e). Tetrahydrofuran **12e** was synthesized from aldehyde **11** (515 mg, 1.28 mmol), 1-bromo-2,4,5-trimethylbenzene (1.28 g, 6.4 mmol), magnesium turnings (155 mg, 6.4 mmol), iodine (10 mg) and THF (20 cm^3) following the procedure described for the synthesis of compound **12c** and the general procudure described for synthesis of compounds **12a**–**12e**. The crude product was purified by column chromatography [3–4% (v/v) MeOH in CH_2Cl_2] to give tetrahydrofuran **12e** (589 mg, 88%) as a colourless oil. R_f 0.34 (CH_2Cl_2–MeOH 95:5, v/v); δ_H ($CDCl_3$) 7.25 (2H, d, *J* 8.7), 7.21 (2H, d, *J* 8.9), 6.90 (1H, s), 6.87 (1H, s), 6.85 (2H, d, *J* 8.9), 6.76 (2H, d, *J* 8.7), 4.95 (1H, dd, *J* 3.6 and 5.9), 4.48 (2H, s), 4.18–4.08 (3H, m), 3.89 (1H, d, *J* 9.6), 3.80 (1H, m), 3.79 (3H, s), 3.77 (3H, s), 3.71 (1H, d, *J* 9.2), 3.64 (1H, d, *J* 2.6), 3.51 (1H, d, *J* 9.4), 3.24 (1H, s), 3.18 (1H, d, *J* 3.4), 2.25 (3H, s), 2.22 (3H, s), 2.21 (3H, s); δ_C ($CDCl_3$) 159.5, 159.3, 136.0, 135.8, 134.2, 132.5, 132.0, 129.8, 129.7, 129.6, 129.5, 128.5, 113.9, 113.8, 88.6, 84.7, 81.7, 75.4, 73.5, 71.7, 70.9, 69.4, 55.3, 19.5, 19.4, 19.0; MALDI-HRMS: *m*/*z* 545.2483 ($[M + Na]^+$, $C_{31}H_{38}O_7Na^+$ calc. 545.2509).

General procedure for the cyclization of compounds 12a–12e to give compounds 13a–13e

N,*N*,*N*′,*N*′-Tetramethylazodicarboxamide (TMAD) was added in one portion to a stirred solution of compounds **12a**–**12e** and tributylphosphine in anhydrous benzene at 0 °C. The mixture was stirred at rt for 12 h, whereupon it was diluted with diethyl ether (50 cm^3). Washing was performed successively with saturated aq. NH_4Cl (2 × 20 cm^3) and brine (25 cm^3), and the separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The crude product obtained was purified by column chromatography [1.5–2% (v/v) MeOH in CH_2Cl_2] to give compound **13a**–**13e**.

(1*S*,3*S*,4*R*,7*S*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (13a). Cyclization of compound **12a** (540 mg, 1.13 mmol) in the presence of TMAD (310 mg, 1.8 mmol), PBu_3 (364 mg, 1.8 mmol) and benzene (10 cm^3) followed by the general work-up procedure and column chromatography afforded compound **13a** as a colourless oil (400 mg, 77%). R_f 0.51 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.36–7.33 (7H, m), 7.10 (2H, d, *J* 8.3), 6.88 (2H, d, *J* 8.7), 6.78 (2H, d, *J* 8.7), 5.17 (1H, s, H-1′), 4.59 (2H, br s, -CH_2(MPM)), 4.43 (1H, d, *J* 11.3, -CH_2(MPM)), 4.34 (1H, d, *J* 11.3, -CH_2(MPM)), 4.19 (1H, s, H-2′), 4.09 (1H, d, *J* 7.7, H-5″), 4.06 (1H, d, *J* 7.7, H-5″), 4.01 (1H, s, H-3′), 3.82–3.77 (5H, m, H-5′, -OCH_3), 3.76 (3H, s, -OCH_3); δ_C ($CDCl_3$) 159.4, 159.3, 139.4 (C-1), 130.3, 129.7, 129.5, 129.3, 128.5, 127.5, 125.4, 113.9, 113.8, 85.9 (C-4′), 84.1 (C-1′), 81.1 (C-2′), 77.4 (C-3′), 73.7 (-CH_2(MPM)), 73.4 (C-5″), 71.8 (-CH_2(MPM)), 66.3 (C-5′), 55.4 (-OCH_3), 55.3 (-OCH_3); MALDI-HRMS: *m*/*z* 485.1948 ($[M + Na]^+$, $C_{28}H_{30}O_6Na^+$ calc. 485.1935).

(1*S*,3*S*,4*R*,7*S*)-3-(4-Fluoro-3-methylphenyl)-7-(*p*-methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-2,5-dioxabicyclo-[2.2.1]heptane (13b). Cyclization of compound **12b** (550 mg, 1.08 mmol) in the presence of TMAD (275 mg, 1.6 mmol), PBu_3 (325 mg, 1.6 mmol) and benzene (10 cm^3) followed by the general work-up procedure and column chromatography afforded compound **13b** as a colourless oil (445 mg, 84%). R_f 0.52 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.28 (2H, d, *J* 8.7), 7.11 (2H, d, *J* 8.6), 7.09–7.08 (2H, m, H-2 and H-6), 6.94 (1H, dd, *J* 8.5 and 9.2, H-5), 6.88 (2H, d, *J* 8.6), 6.79 (2H, d, *J* 8.4), 5.08 (1H, s, H-1′), 4.62–4.55 (2H, m, -CH_2(MPM)), 4.45 (1H, d, *J* 11.1, -CH_2(MPM)), 4.36 (1H, d, *J* 11.6, -CH_2(MPM)), 4.13 (1H, s, H-2′), 4.07, 4.03 (1H each, 2d, *J* 7.6 each, H-5″), 3.99 (1H, s, H-3′), 3.81–3.78 (2H, m, H-5′), 3.80 (3H, s, -OCH_3), 3.77 (3H, s, -OCH_3), 2.23 (3H, d, *J* 1.6, Ar-CH_3); δ_C ($CDCl_3$) 160.7 (d, *J* 238.0, C-4), 159.4, 159.3, 134.8, 134.7, 130.3, 129.6, 129.5, 129.2, 128.5, 128.4, 128.3, 124.3, 124.2, 115.1, 114.8, 113.9, 113.8, 85.9 (C-4′), 83.5 (C-1′), 81.0 (C-2′), 77.1 (C-3′), 73.6 (-CH_2(MPM)), 73.4 (C-5″), 71.8 (-CH_2(MPM)), 66.2 (C-5′), 55.4 (-OCH_3), 55.3 (-OCH_3), 14.7 (d, *J* 3.3, Ar-CH_3); MALDI-HRMS: *m*/*z* 517.1975 ($[M + Na]^+$, $C_{29}H_{31}O_6FNa^+$ calc. 517.1996).

(1*S*,3*S*,4*R*,7*S*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-(1-naphthyl)-2,5-dioxabicyclo[2.2.1]heptane (13c). Cyclization of compound **12c** (700 mg, 1.32 mmol) in the presence of TMAD (345 mg, 2.0 mmol), PBu_3 (405 mg, 2.0 mmol) and benzene (15 cm^3) followed by the general work-up procedure and column chromatography afforded compound **13c** as a colourless oil (526 mg, 78%). R_f 0.53 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.91–7.86 (2H, m), 7.78 (1H, d, *J* 8.2), 7.73 (1H, d, *J* 7.1), 7.53–7.46 (3H, m), 7.32 (2H, d, *J* 8.7), 7.04 (2H, d, *J* 8.7), 6.90 (2H, d, *J* 8.3), 6.71 (2H, d, *J* 8.6), 5.79 (1H, s, H-1′), 4.67–4.61 (2H, m, -CH_2(MPM)), 4.43 (1H, s, H-2′), 4.38 (1H, d, *J* 11.2, -CH_2(MPM)), 4.27 (1H, d, *J* 10.9, -CH_2(MPM)), 4.16 (2H, br s, H-5″), 4.08 (1H, s, H-3′), 3.91, 3.87 (1H each, 2d, *J* 11.0 each, H-5′), 3.81 (3H, s, -OCH_3), 3.72 (3H, s, -OCH_3); δ_C ($CDCl_3$) 159.3, 134.6 (C-1), 133.5, 130.3, 129.8, 129.7, 129.4, 129.3, 128.9, 128.1, 126.4, 125.8, 125.6, 123.8, 122.7, 113.9, 113.7, 85.7 (C-4′), 82.3 (C-1′), 79.9 (C-2′), 78.2 (C-3′), 73.7 (-OCH_2(MPM)), 73.5 (C-5″), 71.8 (-OCH_2(MPM)), 66.3 (C-5′), 55.4 (-OCH_3), 55.3 (-OCH_3); MALDI-HRMS: *m*/*z* 535.2075 ($[M + Na]^+$, $C_{32}H_{32}O_6Na^+$ calc. 535.2091).

(1*S*,3*S*,4*R*,7*S*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-pyren-1-yl-2,5-dioxabicyclo[2.2.1]heptane (13d). Cyclization of compound **12d** (650 mg, 1.08 mmol) in the presence of TMAD (275 mg, 1.6 mmol), PBu_3 (325 mg, 1.6 mmol) and benzene (10 cm^3) followed by the general work-up procedure and column chromatography afforded compound **13d** as a pale yellow solid (496 mg, 79%). R_f 0.53 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 8.29 (1H, d, *J* 8.2), 8.18–8.12 (5H, m), 8.08–8.01 (2H, m), 7.96 (1H, d, *J* 7.5), 7.35 (2H, d, *J* 8.5), 6.97

(2H, d, *J* 8.9), 6.92 (2H, d, *J* 8.8), 6.60 (2H, d, *J* 8.8), 6.09 (1H, s, H-1′), 4.71–4.65 (2H, m, -CH_2(MPM)), 4.49 (1H, s, H-2′), 4.34 (1H, d, *J* 11.4, -CH_2(MPM)), 4.25 (1H, d, *J* 7.3, H-5″), 4.23 (1H, d, *J* 11.1, -CH_2(MPM)), 4.21 (1H, d, *J* 7.8, H-5″), 4.16 (1H, s, H-3′), 3.95–3.94 (2H, m, H-5′), 3.81 (3H, s, -OCH_3), 3.59 (3H, s, -OCH_3); δ_C ($CDCl_3$) 159.4, 159.3, 132.2 (C-1), 131.4, 130.8, 130.7, 130.4, 129.5, 129.4, 128.0, 127.5, 127.4, 126.9, 126.1, 125.6, 125.4, 124.9, 124.8, 124.7, 123.6, 122.0, 113.9, 113.7, 85.9 (C-4′), 82.7 (C-1′), 80.6 (C-2′), 77.9 (C-3′), 73.9 (-OCH_2(MPM)), 73.5 (C-5″), 71.8 (-OCH_2(MPM)), 66.3 (C-5′), 55.4 (-OCH_3), 55.2 (-OCH_3); MALDI-HRMS: *m*/*z* 609.2218 ($[M + Na]^+$, $C_{38}H_{34}O_6Na^+$ calc. 609.2247).

(1*S*,3*S*,4*R*,7*S*)-7-(*p*-Methoxybenzyloxy)-1-(*p*-methoxybenzyloxymethyl)-3-(2,4,5-trimethylphenyl)-2,5-dioxabicyclo[2.2.1]heptane (13e). Cyclization of compound **12e** (550 mg, 1.05 mmol) in the presence of TMAD (275 mg, 1.6 mmol), PBu_3 (325 mg, 1.6 mmol) and benzene (10 cm^3) followed by the general work-up procedure and column chromatography afforded compound **13e** as a colourless oil (425 mg, 80%). R_f 0.52 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.30 (2H, d, *J* 9.0), 7.24 (1H, s, H-6), 7.13 (2H, d, *J* 8.9), 6.89 (1H, s, H-3), 6.88 (2H, d, *J* 8.6), 6.79 (2H, d, *J* 8.6), 5.18 (1H, s, H-1′), 4.64–4.57 (2H, m, -CH_2(MPM)), 4.46 (1H, d, *J* 11.2, -CH_2(MPM)), 4.36 (1H, d, *J* 11.5, -CH_2(MPM)), 4.18 (1H, s, H-2′), 4.14 (1H, s, H-3′), 4.09 (1H, d, *J* 7.9, H-5″), 4.04 (1H, d, *J* 7.7, H-5″), 3.86 (2H, s, H-5′), 3.80 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3), 2.21 (6H, s, 2 × Ar-CH_3), 2.17 (3 H, s, Ar-CH_3); δ_C ($CDCl_3$) 159.4, 159.3, 135.5 (C-1), 134.4, 134.0, 131.7, 131.3, 130.5, 129.9, 129.4, 129.2, 127.2, 113.9, 113.8, 85.6 (C-4′), 82.4 (C-1′), 79.4 (C-2′), 77.6 (C-3′), 73.5 (-OCH_2(MPM)), 73.4 (C-5″), 71.8 (-OCH_2(MPM)), 66.3 (C-5′), 55.4 (-OCH_3), 55.3 (-OCH_3), 19.5 (-CH_3), 19.3 (-CH_3), 18.4 (-CH_3); MALDI-HRMS: *m*/*z* 527.2383 ($[M + Na]^+$, $C_{31}H_{36}O_6Na^+$ calc. 527.2404).

General procedure for the oxidative removal of the *p*-methoxybenzyl groups to give compounds 14a–14e

To a stirred solution of the compound **13a**–**e** in CH_2Cl_2 (containing a small amount of H_2O) at rt was added 2,3-dichloro-5,6-dicyanoquinone (DDQ) which resulted in an immediate appearance of a deep greenish-black colour which slowly faded into pale brownish-yellow. The reaction mixture was vigorously stirred at rt for 4 h. The precipitate was removed by filtration through a short pad of silica gel which was washed with EtOAc. The combined filtrate was washed, successively, with saturated aq. $NaHCO_3$ (2 × 25 cm^3) and brine (25 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The crude product obtained was purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] to give compound **14a**–**14e**.

(1*S*,3*S*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (14a). Compound **13a** (400 mg, 0.86 mmol) was treated with DDQ (600 mg, 2.63 mmol) in a mixture of CH_2Cl_2 (10 cm^3) and H_2O (0.5 cm^3). After the general work-up procedure and column chromatography, compound **14a** was obtained as a white solid material (128 mg, 66%). R_f 0.30 (CH_2Cl_2–MeOH 9 : 1, v/v); δ_H ($(CD_3)_2CO$–CD_3OD; $(CD_3)_2CO$ was added to the compound followed by addition of CD_3OD until a clear solution appeared) 7.40–7.22 (5H, m), 4.99 (1H, s), 4.09 (1H, s), 4.04 (1H, s), 4.01 (1H, d, *J* 7.7), 3.90 (2H, br s), 3.86 (1H, d, *J* 7.7), 3.77 (2H, br s); δ_C ($(CD_3)_2CO$–CD_3OD; $(CD_3)_2CO$ was added to the compound followed by addition of CD_3OD until a clear solution appeared) 140.0, 128.2, 127.2, 125.4, 87.2, 83.7, 83.5, 72.3, 70.2, 58.4; MALDI-HRMS: *m*/*z* 245.0787 ($[M + Na]^+$, $C_{12}H_{14}O_4Na^+$ calc. 245.0784). Crystals were obtained by the following procedure: the white solid material obtained after column chromatography was dissolved in a minimum amount of methanol whereupon CH_2Cl_2 (approx. 10 times the amount of methanol used) was added; the resulting mixture was left at rt for 48 h and then filtered, and the crystals obtained were washed with CH_2Cl_2 and dried under vacuum.

(1*S*,3*S*,4*R*,7*S*)-3-(4-Fluoro-3-methylphenyl)-7-hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (14b). Compound **13b** (400 mg, 0.81 mmol) was treated with DDQ (570 mg, 2.50 mmol) in a mixture of CH_2Cl_2 (10 cm^3) and H_2O (0.5 cm^3). After the general work-up procedure and column chromatography, compound **14b** was obtained as a white solid material (137 mg, 67%). R_f 0.31 (CH_2Cl_2–MeOH 9 : 1, v/v); δ_H (CD_3OD) 7.23 (1H, d, *J* 8.1), 7.19 (1H, m), 6.99 (1H, dd, *J* 8.5 and 9.3), 4.99 (1H, s), 4.09 (1H, s), 4.06 (1H, s), 4.03 (1H, d, *J* 7.6), 3.93–3.91 (3H, m), 2.25 (3H, d, *J* 1.4); δ_C (CD_3OD) 161.9 (d, *J* 243.3), 136.4 (d, *J* 3.4), 129.6 (d, *J* 5.0), 126.1 (d, *J* 22.8), 125.5 (d, *J* 8.0), 115.7 (d, *J* 22.9), 88.5, 85.0, 84.3, 73.5, 71.3, 59.4, 14.5 (d, *J* 3.7); MALDI-HRMS: *m*/*z* 277.0849 ($[M + Na]^+$, $C_{13}H_{15}O_4FNa^+$ calc. 277.0847).

(1*S*,3*S*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(1-naphthyl)-2,5-dioxabicyclo[2.2.1]heptane (14c). Compound **13c** (475 mg, 0.93 mmol) was treated with DDQ (600 mg, 2.63 mmol) in a mixture of CH_2Cl_2 (10 cm^3) and H_2O (0.5 cm^3). After the general work-up procedure and column chromatography, compound **14c** was obtained as a white solid material (170 mg, 67%). R_f 0.31 (CH_2Cl_2–MeOH 9 : 1, v/v); δ_H ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 7.94–7.86 (2H, m), 7.80–7.74 (2H, m), 7.55–7.46 (3H, m), 5.74 (1H, s), 4.56 (2H, br s), 4.37 (1H, s), 4.24 (1H, s), 4.17–4.11 (2H, m), 4.04 (2H, br s); δ_C ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 134.7, 134.0, 130.2, 129.3, 128.6, 126.8, 126.2, 125.8, 123.8, 122.8, 87.4, 83.1, 82.2, 73.1, 71.5, 59.0; MALDI-HRMS: *m*/*z* 295.0943 ($[M + Na]^+$, $C_{16}H_{16}O_4Na^+$ calc. 295.0941).

(1*S*,3*S*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-pyren-1-yl-2,5-dioxabicyclo[2.2.1]heptane (14d). Compound **13d** (411 mg, 0.7 mmol) was treated with DDQ (570 mg, 2.50 mmol) in a mixture of CH_2Cl_2 (10 cm^3) and H_2O (0.5 cm^3). After the general work-up procedure and column chromatography, compound **14d** was obtained as a white solid material (182 mg, 75%). R_f 0.32 (CH_2Cl_2–MeOH 9 : 1, v/v); δ_H ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 8.32 (1H, d, *J* 7.8), 8.23–8.18 (5H, m), 8.06 (2H, br s), 8.01 (1H, d, *J* 7.6), 6.06 (1H, s), 4.47 (1H, s), 4.36 (1H, s), 4.27–4.18 (2H, m), 4.10 (2H, br s); δ_C ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 132.2, 131.0, 128.5, 127.8, 127.3, 126.5, 125.9, 125.7, 125.1, 123.6, 122.1, 87.7, 83.7, 82.6, 73.1, 71.4, 58.9; MALDI-HRMS: *m*/*z* 369.1092 ($[M + Na]^+$, $C_{22}H_{18}O_4Na^+$ calc. 369.1097).

(1*S*,3*S*,4*R*,7*S*)-7-Hydroxy-1-hydroxymethyl-3-(2,4,5-trimethylphenyl)-2,5-dioxabicyclo[2.2.1]heptane (14e). Compound **13e** (355 mg, 0.7 mmol) was treated with DDQ (570 mg, 2.50 mmol) in a mixture of CH_2Cl_2 (10 cm^3) and H_2O (0.5 cm^3). After the general usual work-up procedure and column chromatography, compound **14e** was obtained as a white solid material (120 mg, 65%). R_f 0.31 (CH_2Cl_2–MeOH 9 : 1, v/v); δ_H ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 7.23 (1H, s), 6.92 (1H, s), 5.14 (1H, s), 4.26 (1H, s), 4.10 (1H, s), 4.08, (1H, d, *J* 7.7), 4.00–3.95 (3H, m), 2.23 (6H, s), 2.21 (1H, s); δ_C ($CDCl_3$–CD_3OD; CD_3OD was added to the compound followed by addition of $CDCl_3$ until a clear solution appeared) 135.6, 133.9, 133.8, 131.7, 131.2, 126.6, 86.6, 82.1, 81.9, 72.3, 70.6, 58.5, 19.2, 19.0, 18.1; MALDI-HRMS: *m*/*z* 287.1257 ($[M + Na]^+$, $C_{15}H_{20}O_4Na^+$ calc. 287.1254).

General procedure for dimethoxytritylation of compounds 14a–14e to give compounds 15a–15e

4,4′-Dimethoxytrityl chloride (DMTCl) was added in one portion to a stirred solution of the compound **14a**–**14e** in anhydrous pyridine. After stirring the mixture at rt for 4 h, methanol (0.2 cm^3) was added and the resulting mixture was evaporated to dryness under reduced pressure. The residue was coevaporated successively with anhydrous CH_3CN (2 × 5 cm^3) and anhydrous toluene (2 × 5 cm^3) and then dissolved in CH_2Cl_2 (20 cm^3, acid free after filteration through a short pad of basic alumina). The resulting solution was washed successively with saturated aq. $NaHCO_3$ (2 × 10 cm^3) and brine (10 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The crude product obtained was purified by column chromatography [0.25–0.50% MeOH in CH_2Cl_2 containing 0.5% Et_3N (v/v/v)] affording compound **15a**–**15e**.

(1*R*,3*S*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (15a). Dimethoxytritylation of compound **14a** (108 mg, 0.49 mmol) using DMTCl (214 mg, 0.630 mmol) in anhydrous pyridine (2 cm^3) followed by the general work-up procedure and column chromatography afforded compound **15a** as a white solid material (180 mg, 71%). R_f 0.31 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.66–7.21 (14H, m), 6.84 (4H, d, *J* 8.8), 5.19 (1H, s), 4.29 (1H, s), 4.13 (1H, s), 4.07 (1H, d, *J* 8.4), 4.01 (1H, d, *J* 8.3), 3.78 (6H, s), 3.55 (1H, d, *J* 10.2), 3.50 (1H, d, *J* 10.7), 2.73 (1H, br s); δ_C ($CDCl_3$) 158.6, 149.8, 144.9, 139.4, 136.2, 135.9, 135.8, 130.3, 130.2, 128.5, 128.3, 128.0, 127.6, 126.9, 125.4, 123.9, 113.3, 86.4, 86.0, 83.8, 83.4, 73.0, 71.6, 60.2, 55.3; *m*/*z* (FAB-MS) 525 $[M + H]^+$, 524 $[M]^+$.

(1*R*,3*S*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-3-(4-fluoro-3-methylphenyl)-7-hydroxy-2,5-dioxabicyclo[2.2.1]-heptane (15b). Dimethoxytritylation of compound **14b** (95 mg, 0.38 mmol) using DMTCl (129 mg, 0.42 mmol) in anhydrous pyridine (2 cm^3) followed by the general work-up procedure and column chromatography afforded compound **15b** as a white solid material (126 mg, 61%). R_f 0.32 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.53–7.15 (11H, m), 6.97 (1H, dd, *J* 8.7 and 8.9), 6.84 (4H, d, *J* 8.8), 5.11 (1H, s), 4.26 (1H, d, *J* 3.9), 4.08 (1H, s), 4.03 (1H, d, *J* 8.0), 3.95 (1H, d, *J* 8.0), 3.78 (6H, s), 3.54 (1H, d, *J* 10.5), 3.47 (1H, d, *J* 10.1), 2.26 (3H, d, *J* 1.5), 2.08 (1H, br s); δ_C ($CDCl_3$) 160.8 (d, *J* 244.1), 158.7, 144.9, 135.9, 134.7, 134.6, 130.3, 130.2, 130.1, 128.5, 128.4, 128.3, 128.0, 127.0, 125.2, 124.9, 124.4, 124.3, 115.2, 114.9, 113.4, 86.5, 86.0, 83.7, 83.0, 72.9, 71.7, 60.1, 55.3, 14.8 (d, *J* 3.1); *m*/*z* (FAB-MS) 556 $[M]^+$.

(1*R*,3*S*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(1-naphthyl)-2,5-dioxabicyclo[2.2.1]heptane (15c). Dimethoxytritylation of compound **14c** (125 mg, 0.46 mmol) using DMTCl (170 mg, 0.50 mmol) in anhydrous pyridine (2 cm^3) followed by the general work-up procedure and column chromatography afforded compound **15c** as a white solid material (158 mg, 60%). R_f 0.35 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.95–7.86 (3H, m), 7.79 (1H, d, *J* 8.3), 7.58–7.41 (9H, m), 7.35–7.25 (3H, m), 6.86 (4H, d, *J* 8.8), 5.80 (1H, s), 4.36 (1H, s), 4.32 (1H, d, *J* 6.4), 4.17 (1H, d, *J* 8.3), 4.06 (1H, d, *J* 8.0), 3.78 (6H, s), 3.62–3.56 (2H, m), 2.00 (1H, d, *J* 6.6); δ_C ($CDCl_3$) 158.7, 144.9, 136.0, 135.9, 134.5, 133.6, 130.3, 129.8, 129.0, 128.3, 128.2, 128.1, 127.0, 126.5, 125.9, 125.6, 123.9, 122.6, 113.4, 86.6, 85.7, 82.5, 81.7, 73.1, 72.6, 60.2, 55.3; MALDI-HRMS: *m*/*z* 597.2221 ($[M + Na]^+$, $C_{37}H_{34}O_6Na^+$ calc. 597.2247).

(1*R*,3*S*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-pyren-1-yl-2,5-dioxabicyclo[2.2.1]heptane (15d). Dimethoxytritylation of the compound **14d** (130 mg, 0.38 mmol) using DMTCl (140 mg, 0.42 mmol) in anhydrous pyridine (2 cm^3) followed by the general work-up procedure and column chromatography afforded compound **15d** as a white solid material (147 mg, 61%). R_f 0.37 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 8.46 (1H, d, *J* 8.0), 8.19–8.00 (7H, m), 7.61 (2H, dd, *J* 1.6 and 7.4), 7.48 (4H, d, *J* 8.3), 7.35 (2H, dd, *J* 7.2 and 7.5), 7.25 (1H, m), 7.15 (1H, m), 6.88 (4H, d, *J* 9.0), 6.10 (1H, s), 4.46 (1H, s), 4.43 (1H, br s), 4.25 (1H, d, *J* 8.1), 4.12 (1H, d, *J* 8.1), 3.79 (6H, s), 3.71–3.63 (2H, m), 2.22 (1H, br s); δ_C ($CDCl_3$) 158.7, 149.8, 144.9, 136.1, 136.0, 135.9, 132.1, 131.4, 130.9, 130.6, 130.3, 130.2, 129.2, 129.1, 128.4, 128.3, 128.2, 128.1, 127.5, 127.4, 127.0, 126.9, 126.2, 125.7, 125.5, 125.4,124.9, 124.8, 124.7,123.8, 123.7, 121.9, 113.4, 86.6, 86.1, 83.2, 82.2, 73.2, 72.4, 60.3, 55.3; MALDI-HRMS: *m*/*z* 671.2402 ($[M + Na]^+$, $C_{43}H_{36}O_6Na^+$ calc. 671.2404).

(1*R*,3*S*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(2,4,5-trimethylphenyl)-2,5-dioxabicyclo[2.2.1]heptane (15e). Dimethoxytritylation of compound **14e** (80 mg, 0.30 mmol) using DMTCl (113 mg, 0.33 mmol) in anhydrous pyridine (2 cm^3) followed by the general work-up procedure and column chromatography afforded compound **15e** as a white solid material (134 mg, 78%). R_f 0.32 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H ($CDCl_3$) 7.55 (2H, d, *J* 7.3), 7.45–7.42 (4H, m), 7.32–7.19 (4H, m), 6.93 (1H, s), 6.84 (4H, d, *J* 9.0), 5.20 (1H, s), 4.40 (1H, s), 4.09 (1H, s), 4.04 (1H, d, *J* 7.9), 3.95 (1H, d, *J* 8.5), 3.78 (6H, s), 3.56 (1H, d, *J* 10.7), 3.47 (1H, d, *J* 10.4), 2.24 (3H, s), 2.22 (3H, s), 2.19 (3H, s); δ_C ($CDCl_3$) 158.6, 145.0, 136.0, 135.7, 134.4, 134.2, 131.8, 131.3,130.3, 130.2, 128.3, 128.0, 127.2, 126.9, 113.3, 86.4, 85.7, 82.1, 81.8, 73.0, 71.9, 60.2, 55.3, 19.6, 19.3, 18.4; MALDI-HRMS: *m*/*z* 589.2576 ($[M + Na]^+$, $C_{36}H_{38}O_6Na^+$ calc. 589.2561).

General procedure for synthesis of the phosphoramidite derivatives 16a–16e

2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite was added dropwise to a stirred solution of the nucleoside **15a**–**15e** and *N*,*N*-diisopropylethylamine (DIPEA) in anhydrous CH_2Cl_2 at rt. After stirring the mixture at rt for 6 h, methanol (0.2 cm^3) was added and the resulting mixture was diluted with EtOAc (20 cm^3, containing 0.5% Et_3N, v/v). Washing was performed successively with saturated aq. $NaHCO_3$ (2 × 10 cm^3) and brine (10 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [25–30% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)] to give amidite **16a**–**16e**.

(1*R*,3*S*,4*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-phenyl-2,5-dioxabicyclo[2.2.1]heptane (16a). Treatment of compound **15a** (170 mg, 0.32 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (85 mg, 0.36 mmol) in the presence of DIPEA (0.4 cm^3) and anhydrous CH_2Cl_2 (2.0 cm^3) followed by the general work-up procedure and column chromatography afforded phosphoramidite **16a** as a white solid material (155 mg, 66%). R_f 0.45, 0.41 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_P ($CDCl_3$) 149.3, 148.9.

(1*R*,3*S*,4*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(4-fluoro-3-methylphenyl)-2,5-dioxabicyclo[2.2.1]heptane (16b). Treatment of compound **15b** (95 mg, 0.17 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (53 mg, 0.22 mmol) in the presence of DIPEA (0.3 cm^3) and anhydrous CH_2Cl_2 (2.0 cm^3) followed by the general work-up procedure and column chromatography afforded phosphoramidite **16b** as a white solid material (85 mg, 66%). R_f 0.45, 0.41 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_P ($CDCl_3$) 149.3, 148.8.

(1*R*,3*S*,4*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(1-naphthyl)-2,5-dioxabicyclo[2.2.1]heptane (16c). Treatment of compound **5c** (158 mg, 0.28 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (75.7 mg, 0.32 mmol) in the presence of DIPEA (0.4 cm^3) and anhydrous CH_2Cl_2 (2.0 cm^3) followed by the general work-up procedure and column chromatography afforded phosphoramidite **16c** as a white solid material (127 mg, 60%). R_f 0.47, 0.44 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_P ($CDCl_3$) 149.2, 149.1.

(1*R*,3*S*,4*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(1-pyrenyl)-2,5-dioxabicyclo[2.2.1]heptane (16d). Treatment of compound **15d** (140 mg, 0.22 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (64 mg, 0.27 mmol) in the presence of DIPEA (0.3 cm^3) and anhydrous CH_2Cl_2 (2.0 cm^3) followed by the general work-up procedure and column chromatography afforded phosphoramidite **16d** as a white solid material (124 mg, 68%). R_f 0.51, 0.47 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_P ($CDCl_3$) 149.4, 149.1.

(1*R*,3*S*,4*R*,7*S*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(2,4,5-trimethylphenyl)-2,5-dioxabicyclo[2.2.1]heptane (16e). Treatment of compound **15e** (130 mg, 0.23 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (64 mg, 0.27 mmol) in the presence of DIPEA (0.3 cm^3) and anhydrous CH_2Cl_2 (2.0 cm^3) followed by the general work-up procedure and column chromatography afforded phosphoramidite **16e** as a white solid material (111 mg, 63%). R_f 0.44, 0.42 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_P ($CDCl_3$) 149.0.

Crystallographic data of 14a ‡

$C_{12}H_{14}O_4$, M = 223.23, monoclinic, a = 9.2257(12), b = 6.1948(8), c = 9.5781(12) Å, β = 108.220(2)°, V = 519.96(12) Å^3, space group $P2_1$ (no. 4), Z = 2, D_x = 1.419 g cm^{-3}, $F(000)$ = 236, graphite monochromated Mo-Kα radiation, λ = 0.71073 Å, μ = 0.106 mm^{-1}, T = 120 K. Crystal size 0.43 × 0.15 × 0.12 mm, colourless needles. The intensities of 6577 reflections were measured on a Siemens/Bruker SMART 1K CCD diffractometer to θ_{max} = 29.97° and were merged (R_{int} = 0.0248) to 2705 unique reflections (including Fridel equivalents). Data collection, integration of frame data and conversion to intensities were performed using the programs SMART,[30] SAINT[30] and SADABS.[31] Structure solution, refinement and analysis of the structure, and production of crystallographic illustrations were carried out using the programs SHELXTL[32] and PLATON.[33] The refinement using 201 parameters converged at R_1 = 0.0346 (for $F_o > 4\sigma(F_o)$) and wR_2 = 0.0853 (for all data). The absolute configuration could not be established from this analysis.

Synthesis, deprotection and purification of oligonucleotides

All oligomers 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 **16a**–**16c** (10 min coupling time) and phosphoramidites **16d** and **16e** (20 min coupling time) were >96% and for unmodified deoxynucleoside and 2′-*O*-methylribonucleoside phosphoramidites (with standard coupling time) >99%, in all cases using 1*H*-tetrazole as activator. 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 the oligomers was verified by by MALDI-MS analysis and the purity (>80%) by capillary gel electrophoresis. MALDI-MS *m*/*z* ([M-H]$^-$; found/calc.): **ON2**, 2731/2733; **ON3**, 2764/2765; **ON4**, 2785/2783; **ON5**, 2857/2857; **ON6**, 2775/2775; **ON8**, 3002/3001; **ON9**, 3018/3019; **ON10**, 3094/3093.

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 solution (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0). Concentrations of 1.5 mM of the two complementary strands were used assuming identical extinction coefficients for modified and unmodified oligonucleotides. The absorbance was monitored at 260 nm while raising the temperature 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.

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