Stereoselective Synthesis of (5′*S*)-5′-*C*-(5-Bromo-2-penten-1-yl)-2′-deoxyribofuranosyl Thymine, a New Convertible Nucleoside

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A stereoselective synthesis of (5′*S*)-5′-*C*-(5-bromo-2-penten-1-yl)-2′-deoxyribofuranosyl thymine phosphoramidite is described; the absolute stereochemistry of the compound has been determined by X-ray diffraction analysis. This convertible nucleoside has been incorporated into oligodeoxynucleotides (ODNs) and proved to be suitable for post-synthesis conjugation on solid support. When a diamine is tethered, there is no alteration in the pairing properties of the ODNs. Molecular modelling indicates that the substituent is inserted into the minor groove of the duplex.

Introduction

Functionalized synthetic oligodeoxynucleotide (ODNs) with various ligands are known to play important roles as tools in biological studies and might have chemotherapeutic applications as antisense or sequence-specific artificial nucleases.[1−3] Manipulation of ODNs' properties by tethering other molecules to them implies the introduction of a functionalised linker between the ODN and the molecule of interest. The most popular of these involve the amino function protected with different groups, which may sometimes be troublesome in their use.[4] Non-nucleosidic linkers can be introduced either at the 5′ or at the 3′ end of the ODN without affecting the hybridisation properties of the resulting conjugate, whereas their positioning in the middle of the chain causes destabilisation, raising levels of spot mutation.[5] An alternative that can circumvent the problems of deprotection of the amino function and localisation of the linker, and which also allows both electrophilic or nucleophilic ligands to be tethered, is the use of an electrophilic linker attached to a nucleoside building block: this is the convertible nucleoside approach for ODN conjugates synthesis.[6] Post-synthetic derivatisation of the ODN may occur either in solution or on the solid support. An advantage of carrying out the conjugation chemistry while the polymer is on the solid support is the ease of separation of the product from all the reactants and the potential to recover the unchanged ligand by a simple washing procedure.

We have investigated the stereoselective synthesis of 5′-*C*-substituted thymidine with various alkyl chains bearing electrophilic functions.[7−8] It turned out that, if problems during the preparation of the conjugate and in the synthesis of the phosphoramidite of the convertible nucleoside are to be avoided, the chain needs to be sufficiently long (up to four carbon atoms).

Here we present a stereoselective synthesis of a (5′*S*)-5′-*C*-(5-bromo-2-penten-1-yl)-2′-deoxyribofuranosyl thymine phosphoramidite suitable for automated synthesis and bearing an electrophilic arm at the 5′ carbon of the sugar residue. This new nucleoside permits attachment of functional groups at any point in the oligonucleotide chain without an abasic site.[9] As an example, an ODN has been singly and doubly tethered with a diamine as a nucleophile model. The hybridisation properties of the oligonucleotides were not altered by such a modification. Computer-assisted simulation indicated that the introduced substituent was oriented across the minor groove of the duplex.

Results and Discussion

Synthesis and Stereochemical Assignment

The Sakuraï reaction between 5′-*C*-thymidine aldehyde and allyltrimethylsilane is an efficient process useful in the synthesis of 5′-*C*-substituted-β-D-2′-deoxy-ribofuranosyl-thymines.[10] We previously showed that addition of 5-*tert*-butyldimethylsilyloxy-3-trimethylsilylpentene and 5-bromo-3-trimethylsilylpentene gave nucleosides **1a** and **1c** with tetrahydrofuran rings as major adducts, and linear open forms **2a**, **3a**, and **3c** as minor ones (Scheme 1). Here we have introduced the use of 5-tosyloxy-3-trimethylsilylpentene, which gave relative proportions of the open forms **2b** and **3b** greater than those obtained when the other ω-substi-

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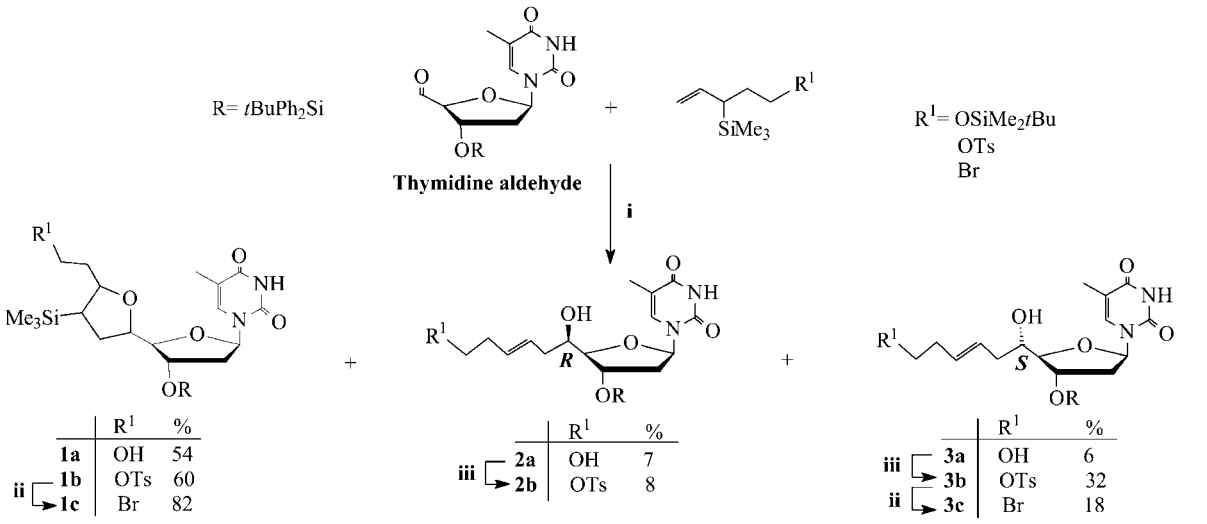
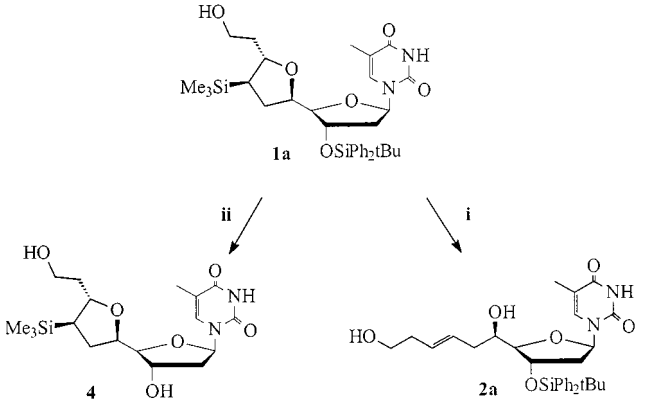


Scheme 1. Reagents and conditions (i) 5 equiv. of 5-substituted 3-trimethylsilylpentene, 5 equiv. of Et_2O-BF_3, CH_2Cl_2, 0° C, 3 h, 60−78%. (ii) LiCl, DMF/acetone, room temp., 18 h, 95%. (iii) TsCl, pyridine, 0° C, 12 h, 90%

tuted 3-trimethylsilylpentenes were used. In order to determine the stereochemical outcome of these reactions, **2a** was converted into **2b**, **3a** into **3b**, and **3b** into **3c**.

Upon treatment with titanium tetrachloride, the silicon moieties in **1a** and **1c** (**1b** had to be brominated before treatment) were eliminated, with concomitant tetrahydrofuran ring-opening to provide **2a**, **3a**, and **3c**. The ratios of 5′-*C*(*R*)/5′-*C*(*S*) after these steps could therefore be determined as 2.8:1, 1:4, and 0:1 for addition of 5-*tert*-butyldimethylsilyloxy-, 5-tosyloxy-, and 5-bromo-3-trimethylsilylpentene, respectively. We assigned the absolute configurations of the 5′ carbons of **2a**, **2b**, and **3a−c** on the basis of an X-ray diffraction analysis of **4**, obtained by removal of the *tert*-butyldiphenylsilyl protecting group on **1a** (Scheme 2).



Scheme 2. Reagents and conditions. (i) $TiCl_4$, CH_2Cl_2, 5 min, 0° C, quant; (ii) nBu_4NF, THF, room temp., 2 h, 90%

The crystal structure of **4** showed the (*R*) absolute configuration at the C-5′ atom (Figure 1). When treated with titanium tetrachloride, **1a** is cleanly converted into **2a**. This allowed (*R*) absolute configurations to be assigned to the 5′ carbons of **2a** and **2b**, and (*S*) configurations to **3a**, **3b**, and **3c**. Other information provided is that the sugar puckering is not altered by the 5′-C substitution and is in the C(2′) *endo* conformation, as usual in the deoxyribonucleosides. This observation at the solid-state level agrees with the values of the $H_{3'}$-$H_{4'}$ coupling constant observed by ^1H NMR for **4** (≈ 3 Hz) and for **2a**, **2b**, and **3a−c** (< 2 Hz), giving an approximation of 70−80% of the population in the 2′*endo* form (% 2′*endo* = 100 − 10·$J_{3'-4'}$).[11] Regarding the furan ring, it can be observed that the O(5′)−C(8′)/C(7′)−Si bonds are in *anti* positions, which is in agreement with the occurrence of an siliranium ion intermediate during the Sakuraï reaction.[12]

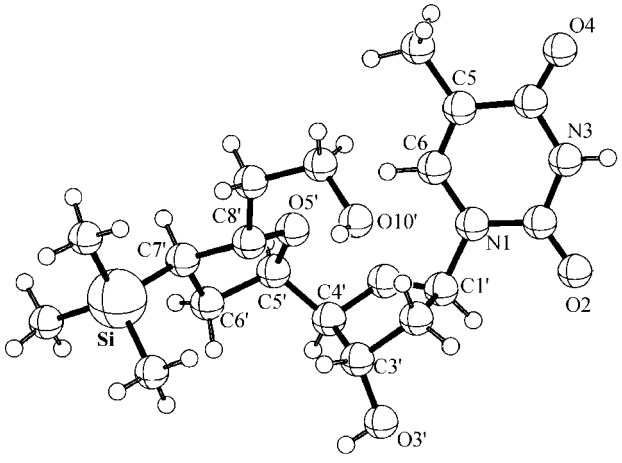
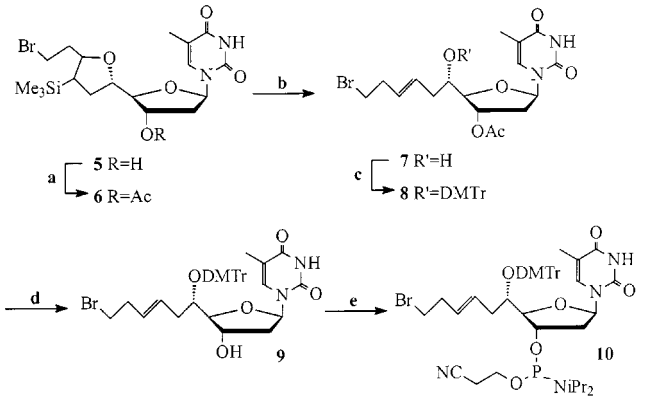


Figure 1. X-ray crystal structure of **4** (ZORTEP drawing with thermal ellipsoids at the 50% probability level)

Since we had chosen to use the phosphoramidite methodology to build ODNs, we needed to introduce a dimethoxytrityl moiety as the protecting group on the 5′-hydroxyl function and a phosphoramidite at the 3′-position (Scheme 3). Compound **1c** is the starting compound best

suited for our purpose, since the electrophilic moiety is already present in the molecule.



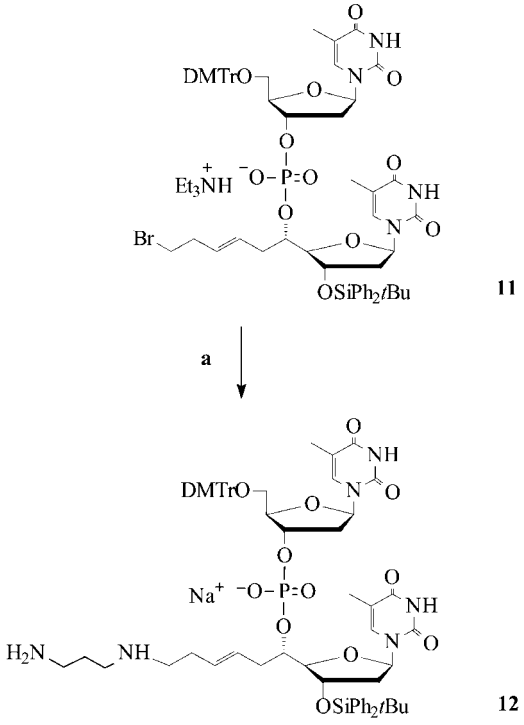
Scheme 3. Reagents and conditions. (a) Ac_2O, Pyr, CH_2Cl_2, room temp., 20 h, 95%; (b) 2 equiv. $TiCl_4$, CH_2Cl_2, 0° C, 15 min, 83%; (c) DMTrCl, $AgNO_3$, collidine, THF, room temp., 12 h, 85%; (d) K_2CO_3, MeOH/H_2O, room temp., 3 h, 98%; (e) (2-cyanoethyl)(*N*,*N*-diisopropylamino)chlorophosphite, Et(*i*Pr)$_2$N, THF, room temp., 35 min, 90%

Removal of the *tert*-butyldiphenylsilyl protecting group at 3′-O turned out to be troublesome when performed at later stages in the synthesis, but in **1c** it could easily be cleaved by fluoride ion to provide **5**. Here, HF/pyridine proved to be superior to tetrabutylammonium fluoride, as use of the latter resulted in substantial loss of material. An acetyl group was then introduced in a very good yield. Compound **6** could be treated with titanium tetrachloride to open the furan ring cleanly, with formation of the unsaturated linear carbon chain bearing the bromine. The 5′-hydroxyl function of **7** was then protected with a dimethoxytrityl group in 85% yield by use of silver nitrate and collidine as previously described for the protection of a secondary hydroxyl function on a *ribo* nucleoside.[13] The acetyl protecting group in **8** was quantitatively removed by potassium carbonate in methanol. Treatment of **9** with chloro(cyanoethoxy)diisopropylaminophosphite provided **10**, suitable for automated synthesis of ODNs, in a very good yield (90%).

Model Study of Conversion

In order to investigate the efficiency of the proposed method to conjugate nucleophiles onto ODNs, we prepared a dinucleoside model by the phosphoramidite method.

Thymidine phosphoramidite was condensed with **3c** in good yield (92% with 5 equiv. of the phosphoramidite), and the crude material obtained was then treated with triethylamine to provide **11** (Scheme 4) with removal of the phosphate protective group (cyanoethyl). The chirality at phosphorus was abolished, allowing easy analysis of the compound. The formation of **11** indicated that there was no problem in use of the phosphoramidite method to build ODNs with 5′-*C*-substituted nucleosides even in the presence of electrophiles on the side chain. Propane-1,3-diamine was used as a model nucleophile, and might also serve as a linker to tether electrophilic molecules. The dinucleoside **11** was mixed in methanol with a large excess of the diamine, and **12** was cleanly obtained as its sodium salt after washing with sodium hydroxide. The functionalized dinucleoside **12** was characterised by ^{1}H and ^{31}P NMR and mass spectrometry.



Scheme 4. Reagents and conditions (a) 10 equiv. of propane-1,3-diamine, MeOH, 50 °C, 2 h, then NaOH 0.5 N, 88%

Oligonucleotides Synthesis and Pairing Properties

A first, simple application of the new convertible nucleoside was to use it as a terminal linker to tether a fluorescent dye. We prepared a 24-mer oligonucleotide primer (5′-T*GCAATTAACCCTCACTAAAGGGA-3′) complementary to the T3 promoter sequence. Phosphoramidite **10** was thus introduced at the last step of the automated synthesis. The oligonucleotide was then deprotected and removed from the solid support with concentrated ammonia; the bromine of the 5′-C side chain of the terminal nucleoside was converted into a primary amine in this step. The crude material was then dried and diluted with a solution of a rhodamine derivative (Texas Red) activated by an *N*-hydroxysuccinimide ester, in formamide. The "blue" oligonucleotide was purified by precipitation in ethanol. Sequencing experiments were run with a Vistra DNA system kit; the primer used proved to be very efficient, regardless of the analysed DNA sequence.

We also prepared two oligonucleotides sequences with one and two modified thymidine moieties (T*). Sequence **A** (5′-GGATTTAT*TGGGTTTAAAGG-3′) and sequence **B** (5′-GGAT**T***TATTGGGT**T***TAAAGG-3′) were synthesised on 50 nmol to 0.2 μmol scales by standard phosphoramidite methodology, except that the coupling time of the phosphoramidite directly following the modified one was increased to ten minutes. On the basis of trityl assays

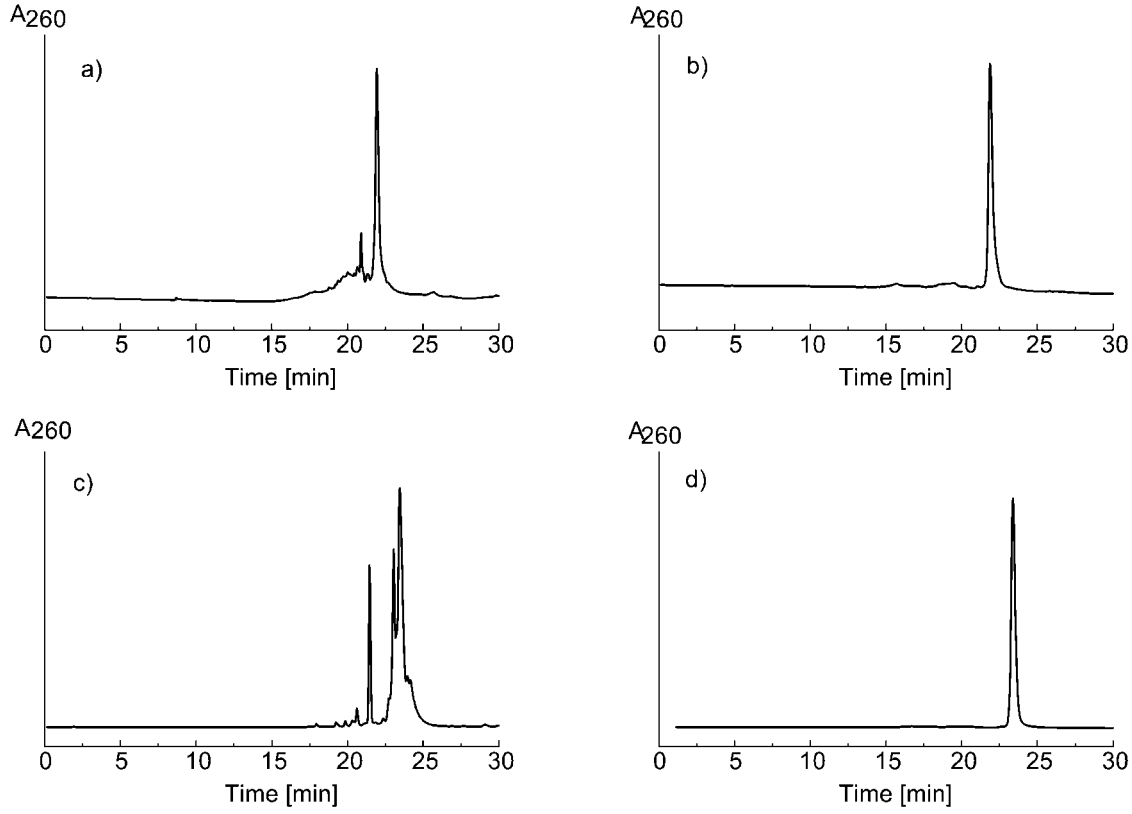


Figure 2. a) and b) HPLC profiles of the crude and purified oligonucleotide **A*** 5′-d(GGATTTA**T***TGGGTTTAAAGG)-3′. c) and d) HPLC profiles of the crude and purified oligonucleotide **B*** 5′-d(GGATTTA**T***TGGGT**T***TAAAGG)-3′. Column: RP 18 prontosil (250 × 4 mm); gradient 0−5 min 100% of I, 5−25 min from 100% of I to 75% of I in II, 25−30 min 75% of I in II (I: TEAA buffer 0.05 M, pH 6.5; II: CH_3CN)

and materials recovered, phosphoramidite **10** was incorporated with the same efficiency as the commercial phosphoramidites. Sequences **A** and **B** were treated, still on solid support, with a solution of propane-1,3-diamine (50%) in anhydrous acetonitrile for 4 hours. The eluent was monitored by UV (λ = 260 nm); no removal of oligonucleotide from the solid support was detected. The oligonucleotides were deprotected and removed from the solid support with concentrated ammonia. After HPLC purification (Figure 2), conjugated sequences **A*** (obtained almost quantitatively) and **B*** (major product) were analysed by mass spectrometry (electrospray).

We found 6381.7±0.5 and 6521.6±0.5, respectively (theoretical values 6382 and 6521). These mass values were greater by 140 and 280, respectively, than those for an unmodified natural identical sequence. The transition temperatures of the duplexes from the amino-functionalized oligonucleotides **A*** and **B*** and the complementary partner strand were determined at pH 6.95 in the presence of 100 mM NaCl. In comparison with the *T*m of the unmodified duplex (57.6 °C), the duplex formed with **A*** (one modification) showed a slight increase of 0.5 °C (*T*m = 58.1 °C), whereas the duplex formed with **B*** (two modifications) had a *T*m decrease by 1.2 °C (*T*m = 56.4 °C).

The methodology developed for the synthesis of 5′-*C*-substituted thymidine is straightforward and versatile. 5′-*C*-Uridine aldehyde reacts in the same way and should allow the production of 5′-*C*-substituted nucleoside in the ribo series. By appropriate choice of the substituted allyltrimethylsilane, it is possible to prepare both of the two stereoisomers at 5′-C. Moreover, the arm with the electrophilic function (bromine) is introduced in a single step starting from the nucleoside aldehyde. It is noteworthy that the double bond present in the alkyl chain can be reduced (at the nucleoside level) to introduce greater flexibility on the modification or can be used to introduce a reactive aldehyde function into the oligonucleotide through a two-step procedure (dihydroxylation/oxidative cleavage). The preparation of the dinucleotide **11** indicates first that the 5′-*C*-substitution does not interfere with the phosphoramidite chemistry used to built oligonucleotides and, on treatment with a diamine, allows the introduction of an amino linker without the need to differentiate the two reactive amino functions. Preliminary studies that we have performed on 5′-tosyloxypropylthymidine showed that pyridinium or aminoguanidinium can also be coupled under the same conditions.[8]

The Δ*T*m values observed for the diamine-oligonucleotide conjugates **A*** and **B*** (+0.5° and −0.6° per modification) should be compared with those observed when aminomethyl (−0.3°), methoxymethyl (−0.6°) and allyl (−1.4°) moieties are introduced as (5′*S*)-5′-*C*-substituents on thymidine[14] and − with more caution, since those modified thymidine were used as 5′-*C* epimeric mixtures − with the values found when methyl (0° to −0.2°) and hydroxymethyl

(−1° to −2°) moieties are introduced.[15−16] The longer the chain length, the greater the destabilisation of hybridisation. The modification introduced by us is composed of ten atoms but there are two ionisable functions (a primary and a secondary amine), and so the negative effect of an alkyl chain can be complemented by ionic interaction between the positively charged amino function and the negative charge of a phosphate. A computational model of a DNA/RNA duplex with the modified thymidine in the middle of the chain was built and minimised in an aqueous medium (Figure 3). It clearly appeared that, with an (*S*) absolute configuration at 5′-C, the substituent is directed into the minor groove of the duplex. In our case, moreover, the aminoalkyl chain is long enough to reach a phosphate in the complementary strand.

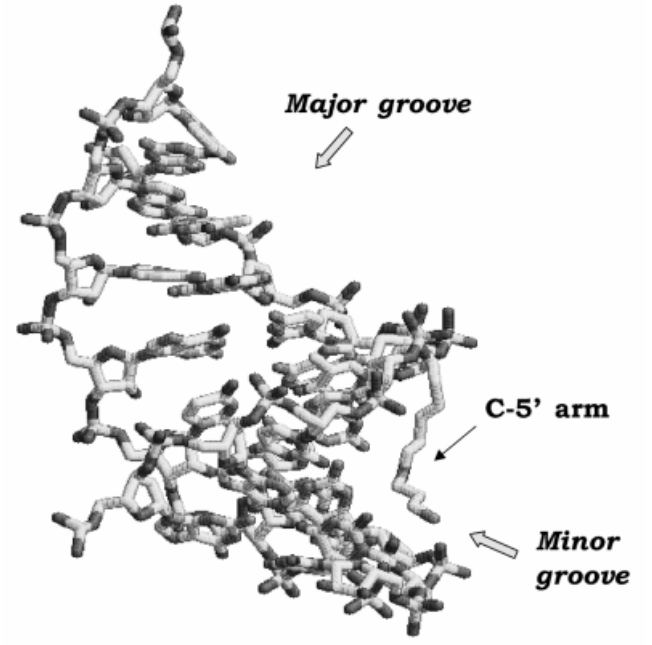


Figure 3. Model representation (Biosym, Insight II) of a DNA/RNA duplex (5′-GACGT*ACGTG-3′)/(3′-CUGCAUGCAC-5′). T* is a thymidine substituted with a 5-(3-aminopropylamino)pentene arm at the 5′-C-position. The duplex was constructed with the substituent, and minimisation was run in a water medium. The attached functional group can potentially pass through the minor groove to reach the other strand.

Conclusion

The 5′-positions in 2′-deoxynucleosides are a new promising site for modification and therefore enable rapid preparation of a wide range of oligonucleotide conjugates. Since the stereochemistry of the new created asymmetric centre can be controlled, interactions either in the major or in the minor groove of a duplex can be effected by adjustment of the absolute configuration at 5′-C. The chemistry described for the introduction of an electrophilic arm at the 5′-*C*-position by means of a Sakuraï reaction with 5-bromo-3-trimethylsilylpentene can be applied to the other nucleoside aldehydes both in the 2′-deoxyribo- and in the ribo series. We are now preparing oligonucleotides tethered with various ligands potentially capable of acting on the phosphodiester linkage of a RNA counterpart in order to increase the chemical diversity of nucleic acids and hence their catalytic potential.[17]

Experimental Section

General Remarks: Products were purified by medium pressure liquid chromatography on a Jobin et Yvon Modoluprep apparatus through Amicon 6−35 μm or Merck 15 μm silica. − IR spectra were recorded with a Perkin−Elmer 883 spectrometer, while Bruker AC 80, AC 200, or AC 250 spectrometers were used for NMR spectra (80 or 250 MHz for ^{1}H, 62.9 MHz for ^{13}C, and 81 MHz for ^{31}P). Chemical shifts were referenced to the tetramethylsilane. − Mass spectra were recorded on a Nermag R10-10 (DCI) or on a Perkin−Elmer API 141 (electrospray). − All solvents were distilled and dried before use.

Compounds 1a, **1c**, **2a**, **3a**, and **3c** are described in ref.[10]

Preparation of 2a from 1a: Titanium tetrachloride (0.105 mL, 0.88 mmol) was added at 0 °C to a solution of **1a** (0.28 g, 0.44 mmol) in anhydrous dichloromethane. After 5 min, a saturated aqueous solution of $NaHCO_3$ (10 mL) was added. The mixture was extracted twice with ethyl acetate, and washed with HCl (1 N, 10 mL) and brine. The organic layer was dried with $MgSO_4$ and concentrated in vacuo. Compound **2a** was obtained as a white foam (0.247 g, 99%). All properties were identical to those observed in ref.[10] (compound **13**).

Compounds 1b, 2b, and 3b: Et_2O/BF_3 (4 mL, 5 equiv.) and 5-tosyloxy-3-trimethylsilylpentene (10.12 g, 5 equiv.) were added at −78 °C to a solution of thymidine aldehyde (3.1 g, 6.48 mmol) in anhydrous dichloromethane (50 mL). The reaction mixture was allowed to come to room temperature over 4 h. The reaction was stopped by addition of a saturated aqueous solution of $NaHCO_3$ (50 mL) and the mixture was extracted twice with ethyl acetate. The organic layers were dried with $MgSO_4$ and concentrated in vacuo. Compounds **1b** (1.84 g, 60%), **2b** (223 mg, 8%), and **3b** (894 mg, 32%), a 60% combined yield from thymidine aldehyde, were isolated after chromatography on silica gel with petroleum ether/dichloromethane/ethyl acetate: 1:3.2:0.8 as solvent.

5-[4′-Trimethylsilyl-5′-(2"-tosyloxyethyl)-1′-oxacyclopent-2′-yl]-4-*tert*-butyldiphenylsilyloxy-2-thyminyl-1-oxacyclopentane (1b): ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = −0.05 (s, 9 H, $SiMe_3$), 0.86 (m, 1 H, H$^{7'}$), 1.08 (s, 9 H, *t*Bu), 1.79 (s, 3 H, Me^{7}), 1.48−2.03 (m, 5 H, H$^{2'b,6',9'}$), 2.35 (m, 1 H, H$^{2'a}$), 2.40 (s, 3 H, CH_3), 3.22 (m, 1 H), 3.48 (m, 1 H), 3.80 (t, 1 H, *J* = 2.7, H$^{4'}$), 4.07 (m, 2 H, H$^{10'}$), 4.34 (m, 1 H, H$^{3'}$), 6.39 (dd, 1 H, *J* = 5.8; 8.0, H$^{1'}$), 7.24−7.28 (m, 4 H, Ph), 7.36−7.46 (m, 7 H, Ph, H^{6}), 7.62−7.74 (m, 6 H, Ph), 8.91 (s, 1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ = −2.7, 12.5, 19.1, 21.6, 26.9, 30.5, 32.0, 35.6, 40.3, 68.1, 78.5, 84.8, 87.5, 110.6, 127.8, 127.9, 127.9, 129.8, 130.1, 133.1, 133.1, 133.3, 135.5, 135.7, 135.8, 144.8, 150.3, 163.8. IR ($CHCl_3$): ṽ [cm^{-1}]: 3194 (NH), 3070 (=CH), 2955−2859 (−CH), 1693(C=O). $C_{41}H_{54}N_2O_8SSi_2$ (791.1): calcd. C 62.25, H 6.88, N 3.54; found C 62.01, H 6.63, N 3.80.

(5′*S*)-3′-*O*-(*tert*-Butyldiphenylsilyl)-5′-*C*-(5′′-tosyloxypent-2′′-enyl)-thymidine (2b): ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 1.08 (s, 9 H, *t*Bu), 1.84 (s, 3 H, Me^{7}), 1.92−2.34 (m, 5 H, H$^{2'b,6',9'}$), 2.42 (s, 3 H, CH_3), 2.52 (m, 2 H, H$^{2'a}$), 3.08 (m, 1 H, H$^{5'}$), 3.76 (s, 1 H, H$^{4'}$), 4.01 (t, 2 H, *J* = 6.5, H$^{10'}$), 4.46 (m, 1 H, H$^{3'}$), 5.32 (m, 2 H, H$^{7',8'}$), 6.30 (m, 1 H, H$^{1'}$), 7.30−7.44 (m, 8 H, Ph), 7.52 (s, 1 H,

H^6), 7.60−7.66 (m, 4 H, Ph), 7.73−7.77 (m, 2 H, Ph), 9.05 (s, 1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ = 12.5, 19.0, 21.6, 26.9, 32.3, 37.6, 39.9, 69.8, 70.2, 74.8, 86.9, 89.1, 110.9, 126.5, 127.8, 127.9, 128.9, 129.8, 129.9, 130.1, 133.1, 133.2, 133.4, 135.7, 135.8, 137.3, 144.9, 150.5, 164.0. $C_{38}H_{46}N_2O_8SSi$ (718.9): calcd. C 63.49, H 6.45, N 3.90; found C 63.80, H 6.03, N 4.08.

(5′*R*)-3′-*O*-(*tert*-Butyldiphenylsilyl)-5′-*C*-(5′′-tosyloxypent-2′′-enyl)thymidine (3b): ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 1.07 (s, 9 H, *t*Bu), 1.84 (s, 3 H, Me^7), 1.88−2.38 (m, 5 H, $H^{2'b,6',9'}$), 2.43 (s, 3 H, CH_3), 2.64 (m, 2 H, $H^{2'a}$), 3.66 (m, 1 H, $H^{5'}$), 3.91 (s, 1 H, $H^{4'}$), 4.02 (t, 2 H, *J* = 6.5, $H^{10'}$), 4.48 (m, 1 H, $H^{3'}$), 5.31 (m, 2 H, $H^{7',8'}$), 6.34 (m, 1 H, $H^{1'}$), 7.30−7.42 (m, 8 H, Ph), 7.52 (s, 1 H, H^6), 7.62−7.67 (m, 4 H, Ph), 7.73−7.77 (m, 2 H, Ph), 8.92 (s, 1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ = 12.5, 19.3, 21.7, 26.9, 32.3, 36.4, 40.6, 70.2, 71.2, 72.9, 86.4, 90.5, 111.1, 127.8, 127.9, 128.2, 129.8. 129.9, 130.1, 135.7, 135.9, 137.2, 144.8, 150.5, 163.8. $C_{38}H_{46}N_2O_8SSi$ (718.9): calcd. C 63.49, H 6.45, N 3.90; found C 63.28, H 6.33, N 3.80.

5-[(2′*R*,4′*R*,5′*S*)-5′-(2"-Hydroxyethyl)-4′-trimethylsilyl-1′-oxacyclopent-2′-yl]-4-hydroxy-2-thyminyl-1-oxacyclopentane (4): Tetrabutylammonium fluoride (180 μL, sol 1 M/THF) was added at room temperature to a solution of **1a** (110 mg, 0.17 mmol) in anhydrous THF (3 mL). After two hours of stirring, the solvent was removed under vacuum and the crude material was submitted to chromatography (silica, ethyl acetate, then ethyl acetate/5% methanol). Compound **4** (58 mg) was obtained and crystallised from a mixture of dichloromethane/petroleum ether. ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 0.03 (s, 9 H, $SiMe_3$), 1.15−1.24 (m, 1 H, $H^{7'}$), 1.55−1.69 (m, 2 H, $H^{9'b,6'b}$), 1.83−1.88 (m, 4 H, CH_3, $H^{9'a}$), 2.10−2.18 (m, 2 H, $H^{2'b,6'a}$), 2.33−2.46 (m, 2 H, OH, $H^{2'a}$), 3.26 (s, 1 H, OH), 3.76 (br. s, 2 H, $H^{10'}$), 3.89 (t, 1 H, *J* = 3.2, $H^{4'}$), 3.96−4.10 (m, 3 H, $H^{5',8'}$), 4.39 (s, 1 H, $H^{3'}$), 6.32 (t, 1 H, *J* = 6.9, $H^{1'}$), 7.39 (s, 1 H, H^6), 9.84 (s,1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ = −2.5, 12.6, 32.7, 33.5, 38.4, 40.5, 60.8, 71.8, 79.1, 81.5, 84.9, 88.9, 111.2, 135.8, 150.8, 164.2. $C_{18}H_{30}N_2O_6Si$ (398.5): calcd. C 54.25, H 7.59, N 7.03; found C 54.01, H 7.82, N 7.15.

5-[(2′*S*)-5′-(2"-Bromoethyl)-4′-trimethylsilyl-1′-oxacyclopent-2′-yl]-4-hydroxy-2-thyminyl-1-oxacyclopentane (5): HF/Pyr (sol 70%, 605 μL) was added to a solution of **1c** (200 mg, 0.28 mmol) in anhydrous THF (3 mL). After one day, the reaction mixture was diluted with ethyl acetate (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$ and the solvent was removed under vacuum. The crude material was filtered through a pad of silica, first with dichloromethane and then with ethyl acetate, to provide **5** (110 mg, 85%) as a white foam after solvent removal. ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 0.05 (m, 9 H, Me_3Si), 1.05 (td, 1 H, *J* = 3.7; 3.1, $H^{7'}$), 1.72−2.24 (m, 8 H, Me, $H^{2'b,9',6'}$), 2.36 (m, 1 H, $H^{2'a}$), 3.51 (t, 2 H, *J* = 6.7, $H^{10'}$), 3.88 (s, 3 H, $H^{4',5',8'}$), 4.43 (m, 1 H, $H^{3'}$), 6.32 (t, 1 H, *J* = 6.7, $H^{1'}$), 7.46 (d, 1 H, *J* = 1.0, H^6). ^{13}C NMR (63 MHz, $CDCl_3$), δ = −2.5, 13.0, 31.4, 31.9, 40.0, 40.9, 73.0, 78.5, 80.5, 85.7, 88.1, 110.9, 136.3, 151.8, 164.9. IR ($CHCl_3$): ṽ [cm^{-1}]: 3610 (OH), 3393 (NH), 3066 (CH), 1685 (C=O). $C_{18}H_{29}BrN_2O_5Si$ (461.4): calcd. C 46.85, H 6.33, Br 17.32, N 6.07; (found) C 46.80, H 6.03, N 6.08.

4-Acetoxy-5-[2′(*S*)-5′-(2"-bromoethyl)-4′-trimethylsilyl-1′-oxacyclopent-2′-yl]-2-thyminyl-1-oxacyclopentane (6): Pyridine (1.1 mL, 13.6 mmol) and acetic anhydride (855 μL, 9 mmol) were added to a solution of **5** (350 mg, 0.75 mmol) in anhydrous dichloromethane (7 mL). The reaction mixture was stirred for 20 hours at room temperature. The solution was concentrated to dryness under vacuum and **6** was obtained as a white foam (363 mg, 95%). ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 0.01 (s, 9 H, $SiMe_3$), 1.90 (s, 3 H, Me), 2.07 (s, 3 H, Me), 1.68−2.20 (m, 5 H, $H^{2'b,6',8'}$), 2.34 (m, 1 H, $H^{2'a}$), 3.47 (m, 2 H, $H^{10'}$), 3.72−4.00 (m, 3 H, $H^{4',5',8'}$), 5.23 (d, 1 H, *J* = 6.0, $H^{3'}$), 6.31 (dd, 1 H, *J* = 9.0, 8.9, $H^{1'}$), 7.46 (s, 1 H, H^6), 8.92 (s, 1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ = −2.6, 12.6, 21.0, 30.4, 31.9, 37.1, 39.4, 42.2, 75.6, 77.7, 80.5, 84.5, 84.9, 111.2, 135.1, 150.8, 164.3, 170.6. IR (KBr): ṽ [cm^{-1}]: 3189 (NH), 3064 (=CH), 2954 (−CH), 1742 (O-C=O), 1693 (C=O). MS (DCI, NH_3): 503 (23%, $[M + H^+]^+$), 520 (94%, $[M + NH_4^+]^+$). $C_{20}H_{31}BrN_2O_6Si$ (503.5): calcd. C 47.71, H 6.21, Br 15.87, N 5.56; found C 48.20, H 5.91, N 5.02.

3′-*O*-Acetoxy-5′-*C*-[(*S*)-5-bromopent-2-enyl]thymidine (7): Titanium tetrachloride (87 μL, 0.79 mmol) was added at 0 °C to a solution of **6** (200 mg, 0.39 mmol) in dry dichloromethane (7 mL). After 15 minutes, the reaction was stopped by addition of an aqueous solution of $NaHCO_3$ (10 mL) and the mixture was diluted with ethyl acetate (50 mL). The organic layer was washed successively with water (10 mL) and brine (5 mL). The aqueous phase was acidified with HCl (1 M solution) until it became clear and was then extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried with $MgSO_4$. Removal of the solvent under vacuum provided **7** as a white foam (140 mg, 83%). ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 1.89 (s, 3 H, Me), 2.07 (s, 3 H, Me), 2.24−2.64 (m, 6 H, $H^{2',6',8'}$), 3.36 (m, 2 H, $H^{10'}$), 3.87 (td, 1 H, *J* = 6.0, 1.5, $H^{5'}$), 3.96 (m, 1 H, $H^{4'}$), 5.33 (m, 1 H, $H^{3'}$), 5.50 (m, 2 H, $H^{7',8'}$), 6.31 (dd, 1 H, *J* = 8.0, 5.5, $H^{1'}$), 7.72 (s, 1 H, H^6), 9.71 (s, 1 H, NH). ^{13}C NMR (63 MHz, $CDCl_3$), δ =: 12.6, 21.1, 33.2, 35.6, 36.8, 37.3, 44.7, 70.5, 76.3; 85.6, 86.2, 111.3, 128.9, 131.4, 136.5, 150.8, 164.2, 170.7. IR ($CHCl_3$): ṽ [cm^{-1}]: 3044−2934 (CH), 1692 (C=O), 1469 (C=C). $C_{17}H_{23}BrN_2O_6$ (431.3): calcd. C 47.34, H 5.38, Br 18.53, N 6.50; found C 47.02, H 5.21, N 6.01.

3′-*O*-Acetoxy-5′-*C*-[(5′*S*)-5-bromopent-2-enyl]-5′-*O*-(dimethoxytrityl)thymidine (8): Compound **7** (140 mg, 0.32 mmol) dissolved with THF (9 mL), collidine (425 μL, 3.2 mmol), and dimethoxytrityl chloride (277 mg, 0.81 mmol in 1 mL of THF) were added at room temperature to a suspension of silver nitrate (136 mg, 0.81 mmol) in anhydrous THF under an inert atmosphere of argon. After one night of stirring, the reaction mixture was diluted with dichloromethane (50 mL) and salts were filtered off. The organic layer was washed with brine (20 mL) and dried with $MgSO_4$. After removal of the solvent, the crude material was subjected to silica gel chromatography (silica 60−200 μm, CH_2Cl_2/Et_3N 2%, R_f = 0.1). Compound **8** (200 mg, 85%) was recovered as a foam slightly contaminated with trityl residue. ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 1.87 (s, 3 H, Me), 1.97 (s, 3 H, Me), 2.13 (m, 2 H, $H^{2'}$), 3.35 (m, 4 H, $H^{6',9'}$), 3.21 (t, 2 H, *J* = 6.8, $H^{10'}$), 3.36 (m, 1 H, $H^{5'}$), 3.73 (s, 6 H, MeO), 3.94 (m, 1 H, $H^{4'}$), 5.13 (m, 3 H, $H^{7',8',3'}$), 6.36 (t, 1 H, *J* = 7.3, $H^{1'}$), 6.79 (m, 4 H, Ph), 7.27 (m, 9 H, Ph), 7.91 (s, 1 H, H^6). ^{13}C NMR (63 MHz, $CDCl_3$), δ = 12.5, 32.7, 35.0, 35.6, 38.2, 55.1, 74.4, 75.1, 83.9, 84.6, 87.5, 111.7, 113.1, 121.1, 126.8, 127.1, 127.6, 127.8, 127.9, 128.1, 128.3, 129.2, 130.3, 135.0, 136.0, 136.4, 145.9, 158.7, 158.7, 164.1, 170.1. IR ($CHCl_3$): ṽ [cm^{-1}]: 3398 (NH), 3011−2896 (CH), 1692 (C=O).

[(5′*S*)-5′-*C*-(5-Bromopenten-2-yl)]-(5′-*O*-dimethoxytrityl)thymidine (9): Potassium carbonate (75.6 mg, 0.54 mmol) was added to a solution of **8** (200 mg, 0.27 mmol) in a mixture of MeOH/H_2O (4:1, 5 mL). After 3 hours stirring, the reaction mixture was diluted with ethyl acetate and washed successively with water and brine. The organic layer was dried with $MgSO_4$ and the solvent was removed under vacuum. Compound **9** was obtained as a white foam (184 mg, 98%). ^{1}H NMR (250 MHz, $CDCl_3$), δ (*J* [Hz]) = 1.81 (s, 3 H, Me), 2.16 (m, 2 H, $H^{2'}$), 3.35 (m, 4 H, $H^{6',9'}$), 3.29 (m, 3 H,

$H^{5',10'}$), 3.77 (s, 6 H, MeO), 3.80 (m, 1 H, $H^{4'}$), 4.35 (m, 1 H, $H^{3'}$), 5.16 (m, 2 H, $H^{7',8'}$), 6.38 (t, J = 6.9 Hz, 1 H, $H^{1'}$), 6.83 (m, 4 H, Ph), 7.29 (m, 9 H, Ph), 7.94 (s, 1 H, H^6). ^{13}C NMR (63 MHz, $CDCl_3$), δ = 12.5, 32.7, 35.1, 35.7, 35.8, 41.2, 55.3, 72.0, 74.2, 84.3, 87.1, 87.3, 111.4, 113.2, 127.1, 127.8, 128.1, 128.6, 130.2, 130.3, 130.4, 135.7, 136.1, 136.5, 146.0, 150.6, 158.7, 164.1. IR ($CHCl_3$): ṽ [cm^{-1}]: 3419 (NH), 2931−2836 (CH), 1691 (C=O). MS (DCI, NH_3): 691 ([M + H^+]$^+$). 710 ([M + NH_4^+]$^+$). $C_{36}H_{39}BrN_2O_7$ (691.6): calcd. C 62.52, H 5.68, N 4.05; found C 62.61, H 5.15, N 4.07.

(5′*S*)-5′-*C*-(5′′-Bromopenten-2-yl)-3′-[(2′′-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite]-5′-*O*-(dimethoxytrityl)thymidine (10): Diisopropylethylamine (458 μL, 2.64 mmol) and (2-cyanoethyl)(*N*,*N*-diisopropylamino)chlorophosphite (318 mg, 1.32 mmol) were added at room temperature, under an inert atmosphere of argon, to a solution of **9** (460 mg, 0.66 mmol) in anhydrous THF (3.15 mL). After 35 minutes, the precipitate was filtered off and the filtrate was diluted with ethyl acetate (50 mL) saturated with argon. The organic layer was washed with a cold saturated aqueous solution of Na_2CO_3 (twice 10 mL) and dried with $MgSO_4$. The solvent was removed under vacuum. The crude material was diluted in a small amount of ethyl acetate and precipitated with cold pentane, filtered, and dried under high vacuum to give **10** (530 mg, 90%). ^1H NMR (250 MHz, C_6D_6), δ (J [Hz]) = 1.05 (2s, 9 H, *t*Bu), 1.23 (m, 2 H), 1.72 (m, 2 H), 1.84 (s, 3 H, Me), 2.12−2.57 (3m, 6 H), 2.87 (m, 2 H), 3.18 (m, 2 H), 3.30 (s, 6 H, MeO), 3.46 (m, 2 H), 4.25 (m, 1 H, $H^{4'}$), 4.69 (m, 1 H, $H^{3'}$), 5.10 (m, 2 H, $H^{7',8'}$), 6.65 (t, 1 H, J = 6.8, $H^{1'}$), 6.76−7.66 (m, 13 H, Ph), 7.95 (s, 1 H, H^6). ^{13}C NMR (63 MHz, C_6D_6), δ = 19.9, 20.0, 22.8, 24.6, 32.4, 32.8, 35.6, 35.9, 40.6, 43.6, 45.4, 54.9, 58.4, 58.7, 73.8, 74.7, 75.1, 84.7, 86.7, 87.7, 111.4, 111.5, 113.6, 127.4, 128.1, 128.7, 130.7, 131.0, 131.0, 136.6, 137.0, 147.0, 151.0, 151.2, 159.4, 164.3. ^{31}P NMR (81 MHz, C_6D_6), δ = −148.9, −148.6. IR ($CHCl_3$): ṽ cm^{-1}: 3419 (NH), 3184 (NH), 2967−2837 (CH), 1693 (C=O). MS (electrospray): 913.3 ([M + Na^+]$^+$), 929.2 ([M + K^+]$^+$).

[(5′*S*)-5′-*C*-(5-Bromopenten-2-yl)-3′-*O*-(*tert*-butyldiphenylsilyloxy)-thymidin-5′-*O*-yl][5′-*O*-(dimethoxytrityl)thymidin-3′-*O*-yl]phosphate (11): Freshly sublimed tetrazole (542 mg, 7.75 mmol), thymidine phosphoramidite (1.15 g, 1.55 mmol) and **3c** (200 mg, 0.31 mmol) were diluted with dry acetonitrile (10 mL), under an inert atmosphere of argon. After 15 minutes stirring, collidine (410 μL, 3.1 mmol) was added, followed by a solution of iodine in H_2O/THF (0.1 M) until the dark colour persisted. The reaction mixture was diluted with ethyl acetate and washed with an aqueous solution of $NaHSO_3$ (5%) until it became clear. The organic layer was collected, washed with water and brine, and dried with $MgSO_4$. The crude material was dissolved in methanol (10 mL) and treated with triethylamine (1 mL) for two hours at 50 °C. After evaporation under vacuum the obtained foam was chromatographed on silica gel (ethyl acetate/methanol: 0 to 20%). Compound **11** was recovered in the form of its triethylammonium salt (350 mg, 85%), as a white foam. ^1H NMR (250 MHz, $CDCl_3$), δ (J [Hz]) = 1.03 (s, 9 H, *t*Bu), 1.15 (t, 9 H, Et_3NH^+), 1.28 and 1.74 (s, 6 H, Me), 2.16−2.49 (3m, 8 H), 2.84 (m, 6 H, Et_3NH^+), 3.24−3.39 (m, 4 H), 3.76 (s, 6 H, OMe), 3.76−3.84 (m, 2 H), 3.92 (s, 1 H), 4.12 (br. s, 1 H), 4.60 (br. s, 1 H), 4.87 (br. s, 1 H), 5.19 and 5.37 (m, 2 H, $H^{7',8'}$), 6.36 and 6.58 (2m, 2 H, $H^{1'}$), 6.79 (d, 4 H, Ph), 7.19−7.63 (m, 20 H, Ph and H^6), 7.90 (br. s, 1 H, H^6), 8.98 and 9.29 (br. s, 2 H, NH), 12.33 (br. s, 1 H, Et_3NH^+). ^{13}C NMR (63 MHz, $CDCl_3$), δ = 8.5, 11.5, 12.5, 18.9, 26.8, 32.4, 36.0, 39.4, 40.2, 45.5, 55.3, 64.1, 75.3, 76.1, 76.5, 84.6, 85.1, 87.0, 87.4, 110.8, 111.3, 113.3, 127.2, 127.8, 128.0, 128.3, 127.9, 128.2, 129.9, 130.0, 130.2, 130.5, 133.4, 133.5, 135.1, 135.4, 135.7, 144.2, 150.9, 158.7, 164.2, 164.4. ^{31}P NMR (81 MHz, $CDCl_3$), δ_{ppm}: −2.05. MS (electrospray < 0): 1231.5−1233.5 (M^-). (electrospray > 0): 1334.5−1336.5 (M + Et_3NH^+ + H^+) 1435.7−1437.7 (M + 2 Et_3NH^+)

[(5′*S*)-5′-*C*-(*N*-Aminopropyl-5-aminopenten-2-yl)-3′-*O*-(*tert*-butyldiphenylsilyloxy)thymidin-5′-*O*-yl][5′-*O*-(dimethoxytrityl)thymidin-3′-*O*-yl]phosphate (12): Propane-1,3-diamine (65 μL, 10 equiv.) was added to a solution of **11** (103 mg, 7.7 10^{-5} mol) in dry methanol (2 mL) and the mixture was stirred for two hours at 50 °C. All the solvents were removed and the obtained foam was dissolved in a solution of NaOH (0.5 N, 2 mL) saturated with NaCl and extracted twice with 100 mL of ethyl acetate 15% methanol. The organic layers were combined and, after removal of the solvent, **12** was obtained as a white powder (85 mg, 88%). ^1H NMR (250 MHz, CD_3OD), δ (J [Hz]) = 1.04 (s, 9 H, *t*Bu), 1.25 (s, 3 H, Me), 1.76−1.95 (m, 7 H), 2.11−2.34 (m, 8 H), 2.70−2.88 (m, 4 H, CH_2N), 3.24 (br. s, 2 H, $H^{5'}$), 3.75 (br. s, 7 H, MeO and 1 H), 3.85 (br. s, 1 H), 3.89 (br. s, 1 H), 4.60 (m, 1 H, $H^{3'}$), 5.37 (m, 2 H, $H^{8',7'}$), 6.26 (m, 1 H, $H^{1'}$), 6.55 (m, 1 H, $H^{1'}$), 6.81−6.85 (m, 4 H, ph DMT), 7.20−7.43 (m, 15 H, ph), 7.58−7.68 (m, 6 H, ph), 7.84 (m, 1 H). ^{31}P NMR (81 MHz, $CDCl_3$), δ: −0.43. MS (electrospray > 0): 1250.5 [M + H^+] (100%).

Oligonucleotide Synthesis and Conjugation: The oligonucleotides were assembled on CPG support (50 nmol to 1 μmol scale) on a Perspective Biosystems 8909 Expedite machine, using standard phosphoramidite chemistry. After complete assembly of the oligonucleotide chain, the fully protected oligonucleotide was treated with the diamine (1.5 mL, 50% in CH_3CN) for 4 h, and removal from the support and complete deprotection were then achieved with NH_4OH (33%) at 55 °C for 2 h. The crude product was analysed and purified by reversed phase HPLC (Prontosil C18) on a Waters apparatus (600 E pomp system controller and a 996 photodiode array detector), using a gradient from 100% of A to 75% of A in B (A: TEAA buffer 0.05 M, pH 6.5; B: CH_3CN). Analyses of the conjugates were performed by mass spectrometry in electrospray mode, with an oligonucleotide concentration of 25 pmol/μL in H_2O/2-propanol/1% Et_3N.

Thermal Denaturation Studies: Absorbance versus temperature profiles were recorded at 260 nm in fused quartz cuvettes on a Hewlett Packard 8453 spectrophotometer equipped with a Peltier temperature control device. Each sample was heated to 90 °C and then slowly cooled before measurements. The temperature was increased by 0.5 °C/min from 20 to 80 °C. The two complementary strands were in 2 μM concentration (phosphate buffer, pH 6.95, 100 mM NaCl), assuming identical extinction coefficient for the 5′-C-substituted oligonucleotide and the corresponding unmodified ones.

X-ray Crystallographic Study of 4: Suitable crystals were obtained by crystallisation from a 1:1 mixture of dichloromethane and petroleum ether. $C_{18}H_{29}N_2O_6Si$; M = 397.52; orthorhombic, $P2_12_12_1$; a = 10.6256(13), b = 7.4416(9), c = 26.012(3) Å; V = 2056.8(4) Å^3; Z = 4; D_x = 1.284 mg/m^3; μ(Mo-K_α) = 0.150 mm^{-1}; 160 K. Structure refinement with SHELXL.97; final R = 0.0296 for 13012 observed reflections. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-147041.Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44−1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Molecular Simulation: Computational results were obtained by using software programs from Molecular Simulations Inc. Calculations were performed with Discover programs using the cvff force field, and graphic displays were generated with the Insight II molecular modelling system on a Silicon Graphics Indigo R 10000. The duplex structure was extracted from the template data bank of the software and the 5′-*C*-[5-(3-aminopropylamino)pentene] arm was added without preliminary minimization. The new structure was minimised in a water layer (5 Å width) with the discover module.

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