

Ferrocenyl-Modified DNA: Synthesis, Characterization and Integration with Semiconductor Electrodes

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Abstract: The ferrocenyl-nucleoside, 5-ethynylferrocenyl-2'-deoxycytidine (**1**) has been prepared by Pd-catalyzed cross-coupling between ethynylferrocene and 5-iodo-2'-deoxycytidine and incorporated into oligonucleotides by using automated solid-phase synthesis at both silica supports (CPG) and modified single-crystal silicon electrodes. Analysis of DNA oligonucleotides prepared and cleaved from conventional solid supports confirms that the ferrocenyl-nucleoside remains intact during synthesis and deprotection and that the resulting strands may be oxidised and reduced in a chemically reversible manner. Melting curve data show that the ferrocenyl-modified oligonucleotides form duplex structures with native

complementary strands. The redox potential of fully solvated ferrocenyl 12-mers, 350 mV versus SCE, was shifted by +40 mV to a more positive potential upon treatment with the complement contrary to the anticipated negative shift based on a simple electrostatic basis. Automated solid-phase methods were also used to synthesise 12-mer ferrocenyl-containing oligonucleotides directly at chemically modified silicon <111> electrodes. Hybridisation to the surface-bound ferrocenyl-DNA caused a shift in the reduction

potential of +34 mV to more positive values, indicating that, even when a ferrocenyl nucleoside is contained in a film, the increased density of anions from the phosphate backbone of the complement is still dominated by other factors, for example, the hydrophobic environment of the ferrocene moiety in the duplex or changes in the ferrocene-phosphate distances. The reduction potential is shifted >100 mV after hybridisation when the aqueous electrolyte is replaced by THF/LiClO₄, a solvent of much lower dielectric constant; this is consistent with an explanation based on conformation-induced changes in ferrocene-phosphate distances.

Keywords: cytidine • DNA • ferrocenyl-nucleoside • molecular films • silicon

Introduction

Recently, several groups, including our own, have demonstrated the attachment of DNA strands to bulk silicon substrates by using electrostatic interactions,^[1] post-synthetic conjugation,^[2,3] or automated solid-phase synthesis.^[4,5] These may provide opportunities for new types of gene sensing^[6–8] based on microelectronics; they may also be useful for the fabrication of nanoscale features using self-assembly processes and be important in the development of DNA-based molecular electronics.^[8–12]

With these concepts in mind we have previously demonstrated the synthesis of regular oligonucleotides at modified silicon electrodes, by generating DNA strands tethered at the 3' terminal.^[5,11] Subsequent STM studies have established that, after hybridisation with complementary strands, the resulting duplex structures effectively lie on the surface.^[4] A challenge now is to seek to functionalise the DNA with appropriate groups and, as a prototypical system we have con-

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 Supporting information for this article is available on the WWW under <http://www.chemeurj.org/> or from the author. HPLC of digested **1-ODN**, UV/Vis spectrum of **1-ODN**, thermal denaturation curve of **1-ODN** with complement, MALDI-TOF spectrum of **1-ODN** and electrochemical analysis of ferrocenyl derivatives.

sidered the synthesis of redox-active DNA strands. For this we and others have focused on the use of ferrocenyl-modified nucleosides and in particular on derivatives in which the redox group is fully conjugated to the nucleobase.^[13,14] Forms of DNA functionalised with redox-active organometallic groups have been developed for some time as artificial nucleases.^[15–18] More recently they have been used for sensing applications,^[6,19–27] and they are also important in studies on DNA-mediated electron transfer.^[5,6,22,28–44] There is also interest in tailoring the properties of DNA for direct use in electronics and photonics applications.^[5,9–11,45–48]

The preparation of DNA oligonucleotides bearing metal-containing groups has involved either post-synthetic conjugation of a preformed complex at the terminal position of an oligonucleotide^[16,38,44,49–51] or addition of metal ions to ligand-modified oligonucleotides.^[15,18,40,52,53] More recently the synthesis of metal-modified nucleosides as phosphoramidite monomers for use in automated solid-phase synthesis, or triphosphates for polymerase substrates have also been explored.^[5,20,23,50,53–62] The advantages of this approach include the ease of synthesis and the ability to incorporate the modified base into the oligonucleotide sequence site-specifically. Furthermore, the former method in particular provides a means for functionalising device features, and surfaces in general, with DNA.^[5]

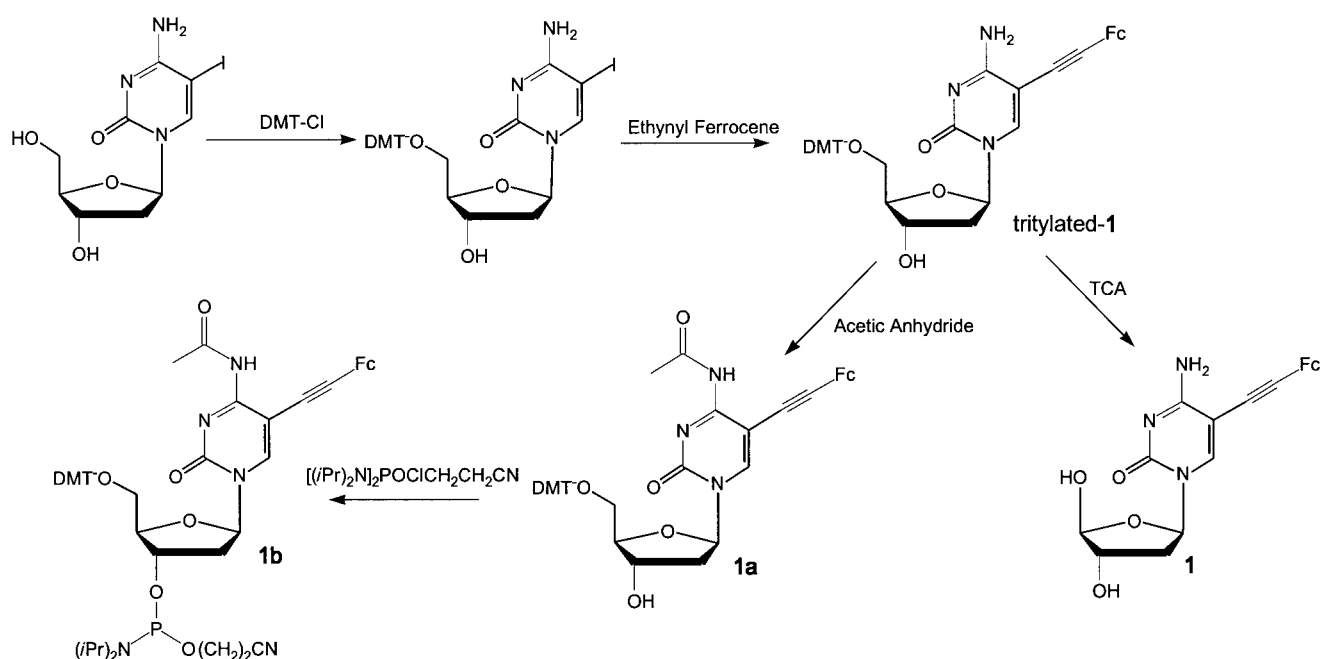
Here we report the synthesis of a new type of metal-modified DNA in which the ferrocenyl group is incorporated into strands at dC sites as 5-ethynylferrocenyl-2'-deoxycytidine, **1**. Oligonucleotide synthesis was performed by using automated solid-phase methods at both conventional control pore glass (CPG) supports, for characterisation in solution, and at modified Si<111> substrates in an effort to assess

the behaviour of these redox-active DNA strands attached directly to a semiconductor electrode surface.

Results and Discussion

Synthesis, spectroscopy and structure of C5-ethynylferrocenylcytidine: The solution-phase synthetic aspects of the work are presented in Scheme 1. Ethynylferrocenyl-deoxycytidine, **1**, was synthesised by Sonogashira cross-coupling of ethynylferrocene and 5-iodo-2'-deoxycytidine. Yields of isolated products and ease of purification were improved when 5-iodo-2'-deoxycytidine was first protected with the dimethoxytrityl (DMT) group due to the increase in solubility of the nucleoside. We have observed similar results previously for C5-ethynylferrocenyl-thymidine.^[14] For subsequent incorporation into oligonucleotides by solid-phase methods, the exocyclic 4-NH₂ group was selectively protected by acetylation by using acetic anhydride in DMF.^[63] Compound **1a** was then converted by reaction with 2-cyanoethoxy-*N,N*-diisopropylaminochlorophosphoramidite to give the fully protected phosphoramidite monomer **1b**.

The electronic absorption spectrum of **1** (Figure 1) shows a shift in the main absorption band to longer wavelength compared to dC ($\lambda_{\text{max}}=291$ nm for **1** compared to $\lambda_{\text{max}}=280$ nm for deoxycytidine). This shift indicates conjugation between the nucleobase and the ferrocenyl group, as has been previously noted for thymidine derivatives.^[14] DFT calculations at the B3LYP level also predict a decrease in the HOMO/LUMO separation for the 1-methyl analogue of **1** (**1-Me**) compared to 1-methylcytosine (1-MeC) ($\Delta E=435$ kJ mol⁻¹ for **1-Me**; $\Delta E=513$ kJ mol⁻¹ for 1-MeC). The HOMO for each is shown in Figure 2 and it can be seen



Scheme 1. Synthesis of C5-ethynylferrocenyl-deoxycytidine, **1**, and its protection for use in solid-phase oligonucleotide synthesis.

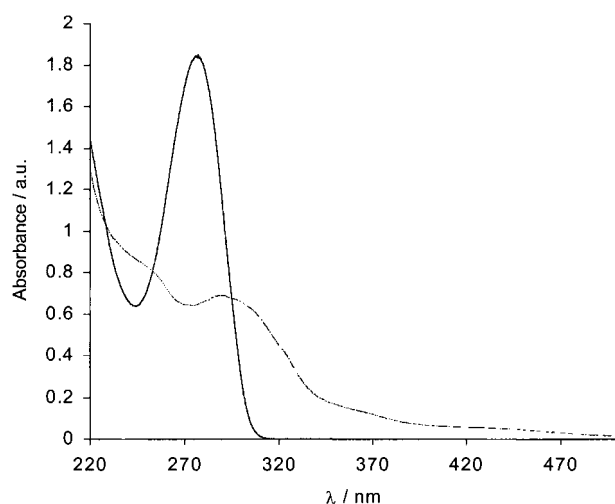


Figure 1. Comparison of the electronic absorption spectra for **1** (grey) and dC (black) in water.

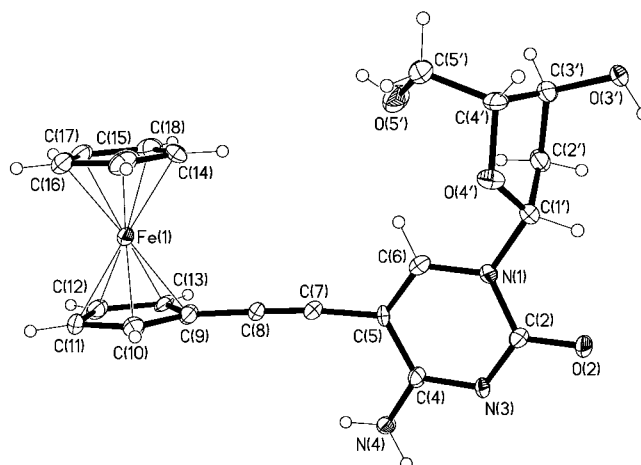


Figure 3. Molecular structure of one independent molecule of **1**. Selected bond lengths [Å] and angles [°]: Fe–C (av) 2.05, Fe–C₅H₅ 1.65, Fe–C₅H₄ 1.66, C(7)–C(8) 1.18, C(8)–C(9) 1.44; Cp–Cp 1.1, interplanar C_p_{sub}–pyrimidine 82.8.

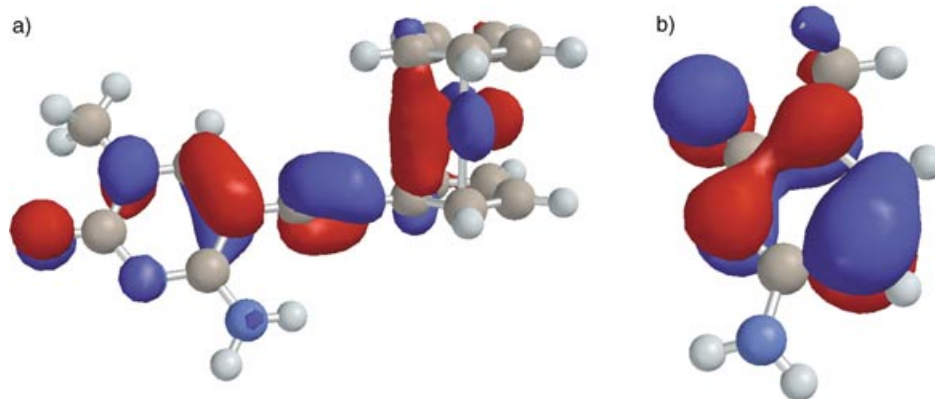


Figure 2. Comparison of the HOMO level of **1**-Me (a) and 1-methylcytosine (b) indicating the delocalisation across the molecule in (a).

that in **1**-Me this molecular orbital extends over much of the molecule.

The molecular structure of C5-ethynyl-ferrocenyl-2'-deoxycytidine, **1**, was obtained by X-ray crystallography of crystals grown by slow evaporation of a dichloromethane/methanol (95:5) solution. The asymmetric unit contains two independent molecules, one of which is shown in Figure 3. All bond lengths and angles lie within the expected range (Fe–C bond lengths range from 2.027(4) to 2.055(4) Å, with an average of 2.044 Å). The Fe–C₅H₅ perpendicular distances are 1.65(4) and 1.65(4) Å in the two molecules, which are similar to the Fe–C₅H₄ distances of 1.66(4) and 1.63(4) Å. The interplanar angles between the cyclopentadienyl rings are 1.1 and 1.2°, and those between the nucleobase and the substituted Cp ring are 82.8 and 87.6°. The two Cp rings within each molecule are virtually eclipsed (mean torsion angles C–X1–X2–C are 2.6 and 2.9°, where X1 and X2 are the centroids of the rings). The ethynyl linkage between the nucleobase and the ferrocene unit has bond lengths indica-

tive of delocalisation, with a central bond length of 1.182(5) and 1.215(6) Å (C(7)–C(8)) and adjacent single bonds C(5)–C(7) of 1.447(5) and 1.412(6) Å, and C(8)–C(9) of 1.440(6) and 1.422(6) Å. This C₂ linkage between the ferrocenyl group and the cytosine moiety deviates only slightly from linearity: C(5)–C(7)–C(8) 174.5(4) and 173.3(4)°, C(7)–C(8)–C(9) 178.3(4) and 177.8(4)°.

Analysis of the crystal packing reveals that all three hydrogen-bonding groups of the nucleobase are involved in intermolecular interactions (Figure 4). A self-complementary base-pairing is seen which involves N(3) of one molecule and the exocyclic amino group N(4) of the second independent molecule (N(3)···N(4) distances of 2.964(5) and 2.929(5) Å). The carbonyl oxygen O(2) interacts with the 3'-OH group (O(2)···HO-3' 2.789(4) and 2.773(4) Å) so as to form an extended parallel network throughout the crystal structure. This packing motif confirms that the attachment of the ferrocenyl moiety to C(5) of the nucleoside does not interfere with its ability to form Watson–Crick-type hydrogen-bonding interactions.

Cyclic voltammetry reveals that **1** exhibits the expected one-electron wave with a formal potential, E^0 , of 290 mV versus ferrocenium–ferrocene (MeCN/NrBu₄PF₆). This is markedly more positive than for the thymidine analogue in the same solvent (E^0 = 140 mV versus Fc/Fc⁺ couple, MeCN/NrBu₄PF₆) and indicates that the nature of the nucleobase has a significant effect on the redox potential, as we

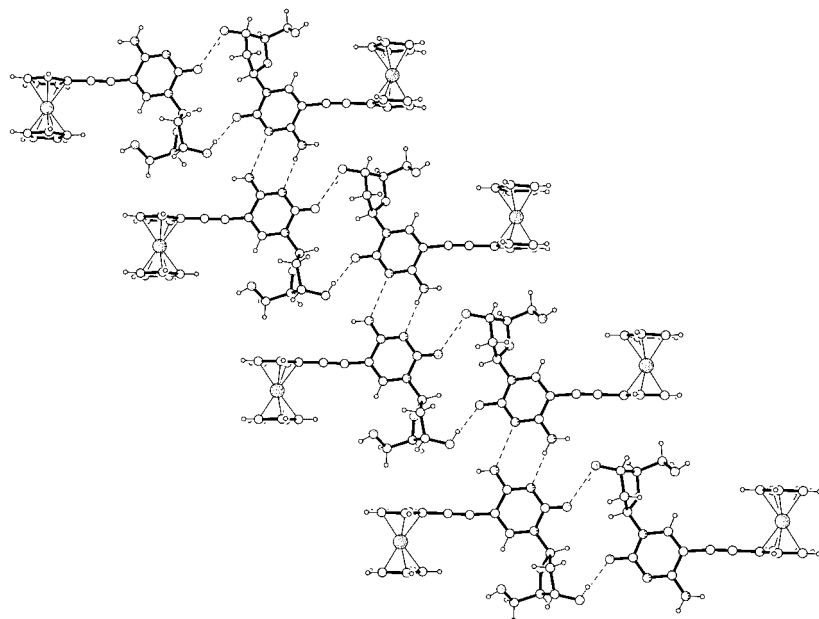


Figure 4. Watson–Crick-type hydrogen-bonding network in the crystal structure of **1**.

have noted for the series of simple ferrocenylmethyl nucleobases.^[66]

Incorporation of C5-ethynylferrocenyl-deoxycytidine, **1, into synthetic oligonucleotides:** It has been noted previously that C5-ethynylferrocenyl-thymidine undergoes base-catalyzed ring closure to the corresponding furanopyrimidone during the deprotection step of regular DNA synthesis.^[29] This involves treatment with aqueous NH_3 for 12 h at room temperature, or for 5 h at 55 °C.^[67] This cyclisation can be reduced, though not eliminated, by use of ULTRAMILD base phosphoramidites (Glen Research, VA, USA).^[14] These reagents are deprotected in a few hours in 0.05 M methanolic K_2CO_3 or in several minutes with anhydrous methylamine. In the case of the cytidine derivative **1**, the deprotection in anhydrous methylamine does not induce cyclisation. As a control experiment, a solution of **1** in CDCl_3 was stirred vigorously in a saturated atmosphere of anhydrous methylamine for 20 min. A ^1H NMR spectrum showed that no cyclisation occurred in this time. The use of anhydrous methylamine is also important to avoid decomposition of the ferrocenium produced in the iodine oxidation steps of solid-phase synthesis. Further, deprotection with anhydrous methylamine does not damage/corrode the silicon wafers/electrodes (vide infra) as can treatments with aqueous NH_3 .^[4]

Oligonucleotide synthesis was performed at commercial control pore glass supports, and at oriented single-crystal $\text{Si} \langle 111 \rangle$ chemically modified for DNA synthesis.^[5] The former solid support allowed analysis of the ferrocenyl-modified DNA by standard methods after cleavage from the surface, whereas the latter offered the opportunity for electrochemical observation of the hybridisation at the semiconductor surface.

The 12-mer oligonucleotide, 5'-AC_{Fc}GTCCAATCGT-3', **1-ODN**, was prepared on 0.2 μmol CPG columns using standard protocols except that, for the insertion of **1**, a prolonged coupling reaction time (15 min) was used.^[57] No attempts were made to optimise coupling efficiency beyond extending the coupling time to 15 min as is common for non-standard phosphoramidites. Deprotection with anhydrous MeNH_2 was monitored by HPLC and it was found that after 10 min the oligonucleotide is fully deprotected. Analysis by MALDI-TOF mass spectrometry confirmed incorporation of the ferrocenyl-nucleoside, **1**, into the oligonucleotide strand (calcd MS [M^+] 3813(± 5); found 3813.98).

HPLC analysis of the purified sequence **1-ODN** after digestion with snake venom phosphodiesterase and alkaline phosphatase (see Supporting Information) confirmed incorporation of **1** (HPLC retention time 32.5 min).

Duplex formation of the ferrocenyl-containing sequence, **1-ODN**, was confirmed by hybridisation with the native complementary sequence, 5'-ACGATTGGACGT-3', **ODN***. Thermal denaturation curves show good reproducibility with a melting temperature indicating a slight reduction in the stability compared to the equivalent unmodified duplex (T_m **1-ODN:ODN*** = 49.4 °C compare **ODN:ODN*** = 52 °C). For single base-pair mismatches against the ferrocenyl-cytidine the following stability sequence was found for 12-mers 5'-ACGATTGGAXT-3': $X=\text{G} > X=\text{T} > X=\text{A} > X=\text{C}$. This can be rationalised from the number of H-bonding interactions, namely $3 \times \text{DA} > 2 \times \text{DA} > 1\text{DD}, 1\text{AA} > 1\text{DD}, 2\text{AA}$ (where D = H-bond donor, A = H-bond acceptor) and supports the expected formation of typical Watson–Crick base-pair formation.

Semi-empirical calculations were performed on the 6-mer oligonucleotides, 5'-AC_xGTCC-3', in an effort to gain further insight into the effect of ferrocenyl incorporation into the duplex. At the PM3 level the converged gas-phase structures indicated that the ethynylferrocenyl group at the C5 position of C_x has little effect on the overall duplex structure. Figure 5 and Figure 6 compare the two optimised structures. The ferrocenyl group is effectively coplanar with the cytidine ring (Figure 5) and is accommodated in the major groove (Figure 6). Local distortions of the modified duplex are minimal, for example, the hydrogen bonding distances for the GC_x pair are equivalent and the dihedral angle between these two bases is 9.8° in the modified case compared to 8.4° for the unmodified. The intra-strand base–base

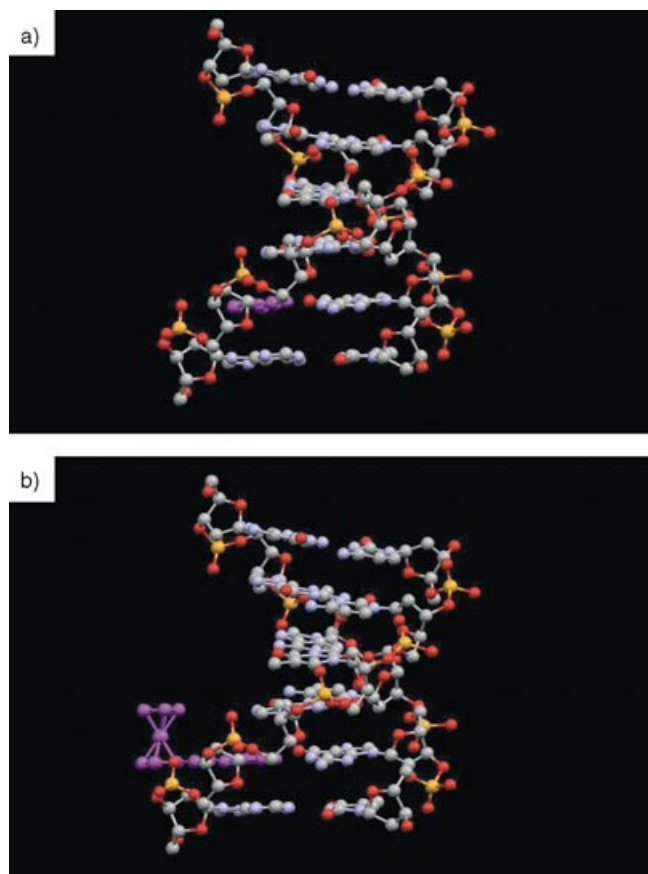


Figure 5. Gas-phase PM3-optimised structures of 6-mer duplexes AC_xGTCC, with a) C_x=cytidine and b) C_x=C5-ethynylferrocenyl-cytidine, showing co-planarity of ferrocene and cytidine ring.

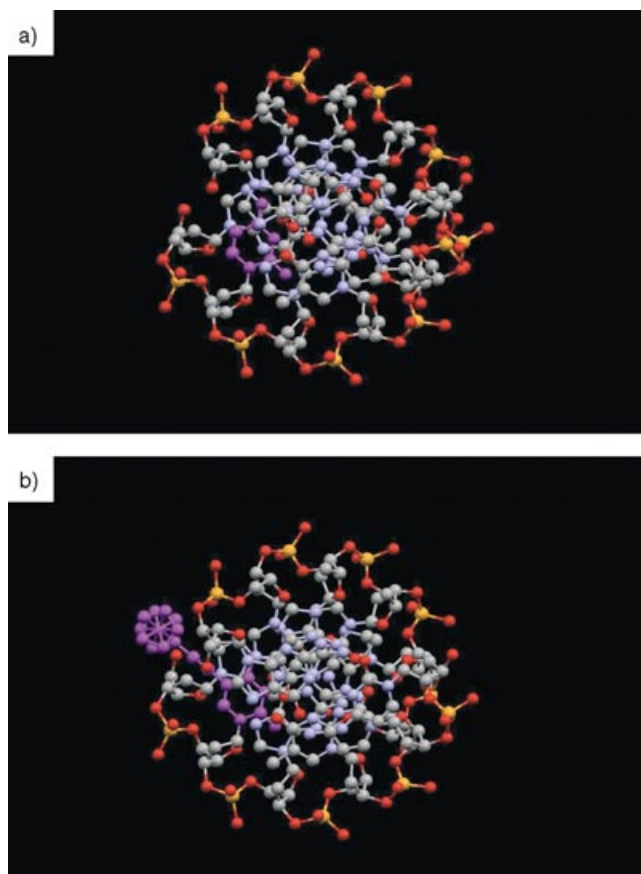


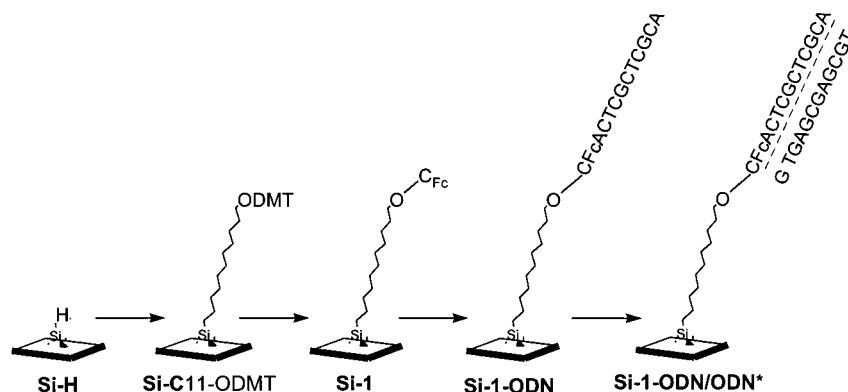
Figure 6. 6-mer duplexes of AC_xGTCC, with a) C_x=cytidine and b) C_x=C5-ethynylferrocenyl-cytidine, viewed along the helical axis, showing the ferrocene moiety sitting external to the phosphate backbone in the major groove.

stacking angles are very slightly affected, with the largest differences ($\sim 2^\circ$) being seen in the complementary 5'-GGACGT-3' strand of the two duplexes. These calculations support the experimental data and suggest that the ferrocenyl-modified oligomers hybridise to complementary sequences with only slight differences from natural strands.

Cyclic voltammetry of the 12-mer **1-ODN** in aqueous solution showed a formal potential of ~ 350 mV (versus SCE) compared to ~ 300 mV for the ferrocenyl nucleoside **1**. A simple electrostatic argument suggests the anionic charge of the phosphodiester backbone would cause a shift to more negative potentials. However, this does not take into account screening by the electrolyte, changes in the ferrocene-phosphate distances and hydrophobic effects associated with the local environment. Upon hybridisation with the complementary strand, we observed a positive shift of $+37$ mV. This suggests that the anions/solvent are capable of screening the extra negative charges on the phosphate groups and that other factors are dominant.

Ferrocenyl-DNA on modified silicon surfaces: The integration of molecular compounds with bulk silicon substrates has expanded significantly since the reports by Chidsey and

Linford on the alkylation of the hydrogen-terminated surface layer.^[68,69] Subsequently a wide range of chemistry has been shown to give similar surface functionalisation with robust Si-C-bonded monolayers.^[70] Our preferred method is thermal hydrosilation of alkenyl/alkynyl derivatives due to its functional group tolerance.^[71,72] Alkylation of hydrogen-terminated Si<111> (**Si-H**) with 4,4'-dimethoxytrityl-protected ω -undecenol provides modified silicon surfaces (**Si-C₁₁-ODMT**) which, after detritylation, present a primary alcohol group suitable for on-chip DNA synthesis.^[5] As a phosphoramidite, **1** was incorporated at the electrode surface as a monomer (**Si-1**) and into growing oligonucleotides (**Si-1-ODN**), as shown in Scheme 2. In these surface bound oligonucleotides the ferrocenyl nucleobase was coupled to the **Si-C₁₁-ODMT** surface as the first nucleobase, the 3'-end. In doing this, the compatibility of **1** with the repeated exposure to reagents used in automated synthesis is rigorously tested. The modified wafers were examined by cyclic voltammetry in both aqueous and organic solvent systems, specifically 1 M LiClO₄ in THF: hybridisation was carried out in aqueous media and the electrode transferred to THF for electrochemistry. It has been shown by electrochemical studies that silicon-bound alkyl monolayers are not penetrated



Scheme 2. Modification of silicon surface with ferrocenyl-cytidine as a monomer, **Si-1**, in single-stranded DNA, **Si-1-ODN** and duplex DNA, **Si-1-ODN/ODN***.

by THF.^[73] In addition these measurements allow some assessment of the role of solvents in charge screening. Table 1 contains data for the ferrocenyl-DNA-modified Si<111> electrodes and the monomer **1** compared to ferrocene in THF solvent.

Table 1. Electrochemical data for ferrocenyl-DNA synthesised at C₁₁OH-modified Si<111> electrode surfaces. The data were measured in 1 M LiClO₄ in THF using a W counter-electrode, Ag quasi reference electrode (Ag QRE). Scan rate 100 mV s⁻¹ (ambient illumination).

Ferrocenyl mono- mer/ modified surface	Anodic peak potential [mV]	Cathodic peak potential [mV]	E^0 [mV]	E^0 after hybridisation [mV]
ferrocene	410	212	311	–
1	181	99	140	–
Si-1	60	0	30	–
Si-1-ODN	32	–2	15	130

Modified Si<111> electrodes bearing ethynylferrocenyl-cytidine, **Si-1**, showed reversible electrochemical behaviour with a formal potential of 30 mV. This is significantly shifted, by –110 mV, to a lower potential in comparison to the free monomer (E^0 = 140 mV). To establish if this was a general phenomenon, the effect of surface attachment on the reduction potential of ethynylferrocene was determined. The same 110 mV difference was observed, ethynylferrocene in THF/LiClO₄ (E^0 = 450 mV) and when attached to Si<111> by thermal alkylation (E^0 = 340 mV).

Figure 7 compares surfaces **Si-1** and **Si-1-ODN** (Si-C_{Fc}ACTCGCTCGCA), and shows there is a small negative shift in the formal potential (–15 mV) upon attachment of further nucleotides. This is in

contrast to solution-based measurements, where a positive shift (+50 mV) was seen upon incorporation of **1** into the polynucleotide chain. This suggests that there is some stabilization of the cationic ferrocenium on the surface, which may be attributed to properties of the bulk film, that is, neighbouring DNA strands, rather than a molecular property of individual DNA chains.

The feasibility of synthesising polyfunctional DNA strands directly at the electrode surface

was explored with surfaces **Si-1-ODN**, **Si-1,1-ODN** and **Si-1,1,1-ODN**. In these the ferrocenyl-cytidine replaces deoxycytidine in the sequence 3'-CACTCGCTCGCA-5'. Integration of the anodic peak current of cyclic voltammograms for these three surfaces shows the expected proportionality between the charge passed during electrochemical oxidation of **Si-1-ODN**, **Si-1,1-ODN** and **Si-1,1,1-ODN** and the number of ferrocene groups (one, two or three C_{Fc} units) incorporated in the sequence (Figure 8). This indicates that the ferrocenyl groups remain intact throughout the repeated cycles of the automated synthesiser. The use of anhydrous methylamine is important to achieve the stability of the incorporated ferrocene groups, which may be oxidised during the solid-phase synthesis and are then susceptible to hydrolysis on exposure to aqueous base.

The complementary sequence was hybridised to the surface-bound ferrocenyl oligonucleotide, **Si-1-ODN**. Cyclic voltammetry performed in HEPES–NaCl buffer indicated a positive shift of +34 mV upon hybridisation. This shift is comparable to the +37 mV observed upon hybridisation of the dissolved ferrocenyl oligonucleotide, **1-ODN**. This suggests that the effect of hybridisation on the redox potential

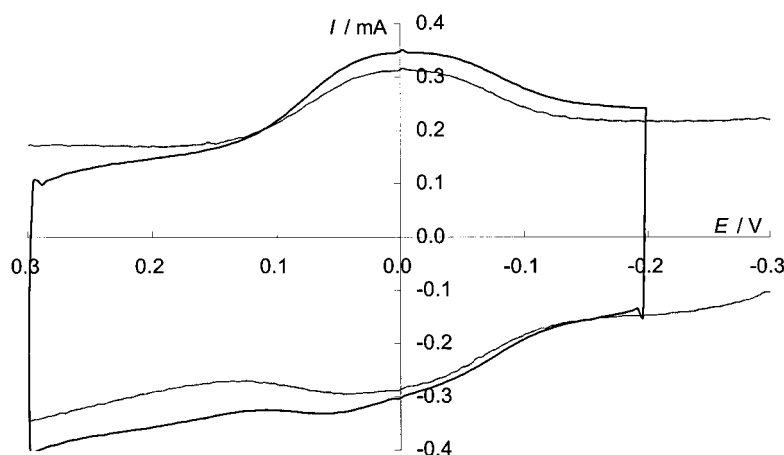


Figure 7. Cyclic voltammograms of **Si-1** (black line) and **Si-1-ODN** (grey line). Electrolyte 1 M LiClO₄ in THF using a Pt counter-electrode, Ag quasi reference electrode (Ag QRE). Scan rate 100 mV s⁻¹.

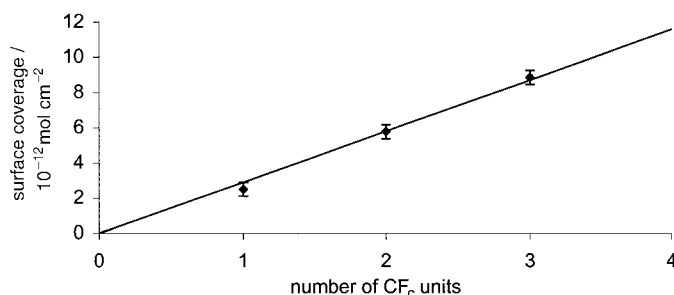


Figure 8. Plot of surface coverage versus number of ferrocenyl groups (CF_c units) in the 12-mer oligonucleotide strands, **Si-1-ODN**, **Si-1,1-ODN** and **Si-1,1,1-ODN**.

has a common origin for both dissolved and surface-bound DNA. In principle, an electrostatic effect can be due to additional charges on the phosphate groups of the complementary strand or a conformational change which alters the ferrocene-nearest phosphate distance; a positive shift can occur from the latter if the extra negative charges are screened equally in both cases.

The same electrochemical comparison was performed by using THF as solvent—the low dielectric constant of THF was expected to enhance electrostatic effects—and gave some interesting results. The observed redox potential shift of **Si-1-ODN** upon hybridisation was +130 mV, a large increase over the aqueous system (+34 mV). Although the simple anionic charge density argument predicts that the redox potential will shift to more negative values, we observe a positive shift in both solvent systems. A hydrophobic effect in which a similar magnitude (40 mV) positive shift in potential upon binding of metal complexes to DNA from aqueous solution is known.^[74] In THF, the hydrophobic effect seems less likely; an alternative explanation is that the shift reflects an increase in the distance between the ferrocene and the nearest phosphate. Our recent STM images of DNA-modified Si<111> surfaces show evidence for conformational changes after hybridisation.^[4] For single-stranded surfaces, little in the way of DNA-related features are apparent. However, upon hybridisation the images feature worm-like structures that are interpreted as regions of aligned DNA molecules lying nearly parallel to the surface. These data are in agreement with recent studies by Barton of the structural dependence of DNA-modified surfaces on the site of attachment.^[75] It is also conceivable that the THF/LiClO₄ electrolyte has a strong influence on the conformation of ssDNA and dsDNA on the surface. To summarise, the positive redox potential shifts upon hybridisation in THF or water are consistent with an explanation in terms of a conformation change-induced increase in the phosphate-ferrocene nearest-neighbour distances.

Conclusion

In contrast to C5-ethynylferrocenyl-thymidine, the cytidine analogue **1** can be incorporated into synthetic DNA oligonu-

cleotides by using phosphoramidite chemistry without transformation. This enables the synthesis of ferrocenyl-modified DNA at standard CPG supports for studies in solution after cleavage, or directly at modified silicon electrodes for surface-confined electrochemistry. The oligonucleotides exhibit the reversible redox behaviour typical of the ferrocenyl group and hybridise with complementary strands. In solution the hybridisation shifts the redox potential of the ferrocenyl group slightly positive (+37 mV). When confined to an electrode by solid-phase synthesis at modified Si<111> wafers the effect of solvent on the shift in redox potential after hybridisation is observable. The silicon-bound DNA films show a shift of +34 mV upon hybridisation with complementary strands when measured in aqueous buffer, but this is enhanced on changing the solvent to THF where the shift in redox potential is +130 mV. The overall increase in the ferrocenyl redox potential indicates that electrostatic effects expected from the increase in anionic charge density from the phosphate groups of the complement (negative shift) are dominated by changes in the conformation which influence the local electronic environment through, for example, increased phosphate-ferrocene distances (positive shift).

Experimental Section

Materials: Reagents were purchased from Aldrich and Lancaster and used as received unless otherwise stated. Solvents were dried and distilled under N₂ prior to use. All reactions were performed under N₂ using standard Schlenk techniques. ¹H NMR spectra were performed on a 200 MHz Bruker Spectrospin AC 200E spectrometer and ³¹P NMR spectra on a 300 MHz Bruker Spectrospin WM 300 WB spectrometer. UV/Vis electronic spectra were recorded on a Shimadzu UV-2101PC scanning spectrophotometer. Mass spectra were measured at the MS Service Centre, University of Wales, Swansea and at the Moredun Research Institute, Scotland.

5'-Dimethoxytrityl-5-iodo-2'-deoxycytidine: 5-Iodo-2'-deoxycytidine (2.0 g, 5.61 mmol) was dissolved in dry pyridine (50 mL) and 4-dimethylaminopyridine (DMAP, 70 mg) and dimethoxytrityl chloride (2.5 g, 7.39 mmol) were added. The mixture was stirred at ambient temperature under nitrogen for 16 h. Then 5 mL of 5% (v/w) sodium bicarbonate was added before the reaction mixture was evaporated to dryness. Final traces of pyridine were removed by repeated dissolution in toluene and evaporating to an oil. The reaction mixture was then dissolved in dichloromethane, washed twice with 5% (v/w) sodium bicarbonate and then dried over magnesium sulfate. After filtration, removal of the solvent gave a thick off-white oil. This was dissolved in the minimum amount of dichloromethane, and methanol was added until the solution went cloudy. Crystallisation overnight at 4°C gave a white powder, which was filtered off and dried at a vacuum pump. Second and third crystallisations yielded further crops of the desired product (1.93 g, 53%). ¹H NMR (200 MHz, [D₆]DMSO): δ = 2.18–2.26 (m, 2H; H-2', H-2''), 3.28 (m, 2H; H-5', H-5''), 3.28 (s, 6H; OMe), 4.01 (m, 1H; H-4'), 4.25 (m, 1H; H-3'), 6.21 (t, 1H; H-1'), 7.01 (d, 4H; DMT), 7.32–7.52 (m, 9H; DMT), 8.07 ppm (s, 1H; H-6); MS: ES +/([M + Na]) = 678.1071, calculated for C₃₀H₃₀N₃O₆INa = 678.1077.

5'-Dimethoxytrityl-5-ethynylferrocene-2'-deoxycytidine, tritylated-1: 5'-Dimethoxytrityl-5-iodo-2'-deoxycytidine (1.0 g, 1.5 mmol) was dissolved in dry acetonitrile (10 mL). Triethylamine (10 mL), ethynylferrocene (480 mg, 2.2 mmol), copper(I) iodide (100 mg, 0.52 mmol), and bis(triphenylphosphine)dichloropalladium(II) (100 mg, 0.14 mmol) were added sequentially under nitrogen. The reaction mixture was stirred at 70°C for

2 h. Disodium EDTA (5% v/w) (5 mL) was added to the resulting suspension before evaporation to dryness. The crude product was redissolved in chloroform (100 mL) and washed twice with disodium EDTA (5% v/w) and once with water before being dried over sodium sulfate. After filtration and concentration by rotary evaporation the reaction mixture was loaded onto a silica gel column packed in chloroform–methanol–triethylamine (95:4:1) and eluted by using chloroform–methanol (95:5). Fractions containing the product were combined and the solvent was removed to yield the title compound as a dark orange powder (954 mg, 86%). ¹H NMR (200 MHz, CDCl₃): δ = 2.18–2.77 (m, 2H; H-2', H-2''), 3.34 (m, 2H; H-5', H-5''), 3.73 (s, 6H; OMe), 4.06 (s, 5H; ferrocene), 4.16 (m, 3H; H-4', ferrocene), 4.23 (m, 2H; ferrocene), 4.47 (m, 1H; H-3'), 6.34 (t, 1H; H-1'), 6.82 (m, 4H; DMT), 7.18–7.45 (m, 9H; DMT), 8.12 ppm (s, 1H; H-6). Elemental analysis calculated (%) for tritylated-1-MeOH: C 67.10, H 5.63, N 5.46; found: C 66.99, H 5.08, N 5.17; MS: ES +/([M + Na]) = 760.2076, calcd for C₄₂H₃₉N₃O₆FeNa = 760.2086.

N-4'-Acetyl-5'-dimethoxytrityl-5-ethynylferrocene-2'-deoxycytidine, 1a: 5'-Dimethoxytrityl-5-ethynylferrocene-2'-deoxycytidine (200 mg, 2.7 mmol) was dissolved in dry DMF (5 mL). Distilled acetic anhydride (33 mg, 3.3 mmol) was added and the reaction mixture stirred at room temperature for 16 h. The reaction was checked by TLC and two more portions of acetic anhydride were added until the reaction had gone to completion. The solvents were removed by rotary evaporation and the resulting orange oil was redissolved in dichloromethane. The mixture was washed with water and dried over magnesium sulfate. The crude product was obtained as a red-brown powder. Purification on a silica column eluted with chloroform–methanol (95:5) with a trace of triethylamine gave the pure desired product (150 mg, 71%). ¹H NMR (200 MHz, CDCl₃): δ = 2.07–2.32 (m, 2H; H-2', H-2''), 2.72 (s, 3H; -OCH₃), 3.35 (m, 2H; H-5', H-5''), 3.72 (s, 6H; OMe), 4.03 (m, 1H; H-4'), 4.13 (s, 5H; ferrocene), 4.20 (m, 2H; ferrocene), 4.25 (m, 2H; ferrocene), 4.52 (m, 1H; H-3'), 6.27 (t, 1H; H-1'), 6.78 (m, 4H; DMT), 7.17–7.40 (m, 9H; DMT), 8.30 ppm (s, 1H; H-6); MS: ES +/([M + H]) = 780.2374, calcd for C₄₄H₄₁N₃O₇Fe = 780.2372.

N-4'-Acetyl-5'-dimethoxytrityl-3'-cyanoethyl-diisopropyl-5-ethynylferrocene-2'-deoxycytidine, 1b: N-4'-Acetyl-5'-dimethoxytrityl-5-ethynylferrocene-2'-deoxycytidine (365 mg, 0.47 mmol) was dissolved in dry dichloromethane (25 mL) and cooled in an ice/salt bath. Freshly distilled diisopropylamine (210 µL, 0.56 mmol) was added, followed by 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (130 µL, 0.3 mmol). The reaction mixture was stirred at 0°C for 1 h and then allowed to warm to room temperature. The product was precipitated by slow addition of the crude reaction mixture, in the minimum amount of dichloromethane, to rapidly stirring hexanes at –78°C. Filtration yielded an orange solid (413 mg, 92%). ¹H NMR (200 MHz, CDCl₃): δ = 1.02–1.11 (m, 12H; NMe₂), 2.03–2.17 (m, 1H; H-2'), 2.24 (t, 2H; CH₂CN), 2.55 (t, 2H; OCH₂CH₂), 2.56 (s, 3H; OCH₃), 2.65–2.81 (m, 1H; H-2''), 3.17–3.21 (m, 2H; H-5', H-5''), 3.23–3.44 (m, 2H; CH(CH₃)₂), 3.54 (d, 6H; OMe), 3.91 (t, 1H; H-3'), 3.97 (s, 5H; ferrocene), 4.03 (m, 2H; ferrocene), 4.11 (m, 2H; ferrocene), 4.25 (m, 1H; H-4'), 6.10 (m, 1H; H-1'), 6.58–6.64 (m, 4H; DMT), 7.04–7.24 (m, 9H; DMT), 8.13 ppm (d, 1H; H-6); ³¹P NMR (300 MHz): δ = 145 (d, P; 150.28, 149.69); MS: ES +/([M + H]) = 980.3460, calcd for C₅₃H₅₉N₅O₈PFe = 980.3450.

5-Ethynylferrocene-2'-deoxycytidine, 1: 5'-Dimethoxytrityl-5-ethynylferrocene-2'-deoxycytidine (50 mg, 0.14 mmol) was dissolved in dichloromethane (10 mL). Several drops of trichloroacetic acid in dichloromethane (2%) were added and the reaction mixture stirred at room temperature until the entire dimethoxytrityl group had been removed as shown by TLC. The solution was concentrated and purified on a short silica column, eluting first with dichloromethane to remove the fast running trityl group and then with dichloromethane–methanol (90:10) to obtain the title compound. ¹H NMR (200 MHz, [D₆]DMSO): δ = 2.14–2.27 (m, 2H; H-2', H-2''), 3.73 (m, 2H; H-5', H-5''), 4.36 (s, 5H; ferrocene), 4.42 (m, 2H; ferrocene), 4.70 (m, 2H; ferrocene), 5.25 (t, 1H; H-3'), 5.237 (d, 1H; H-4'), 6.24 (t, 1H; H-1'), 8.32 ppm (s, 1H; H-6).

Crystal data: 1: C₂₁H₂₁FeN₃O₄·CH₂O, *M*_r = 467.3, triclinic, space group *P*1, *a* = 10.0474(6), *b* = 10.0553(6), *c* = 11.1393(7) Å, α = 72.691(2), β = 77.152(2), γ = 87.676(2)°, *V* = 1047.14(11) Å³, *Z* = 2, *T* = 160 K. Data were

collected on a Bruker SMART 1 K CCD diffractometer with MoK_α radiation (λ = 0.71073 Å, θ = 28.6°) and corrected semi-empirically for absorption, based on repeated reflections: 9056 measured data, 8153 unique, *R*_{int} = 0.0194, transmission factors 0.793–0.949. The structure was solved by direct methods and refined on all *F*² values, with anisotropic displacement parameters and with constrained/restrained isotropic H atoms. The asymmetric unit is the complete unit cell, and contains two molecules of the complex and two molecules of methanol; H atoms were not located for the solvent molecules. *R* (on *F* values for 7123 data with *F*² > 2σ) = 0.0338, *R*_w (on all *F*² values) = 0.0825, goodness-of-fit on *F*² values = 0.984, with 583 refined parameters and 10 restraints. The absolute configuration was confirmed by successful refinement of the Flack parameter to 0.002(13).^[63] The final difference map contained no features outside ±0.42 e Å^{–3}. Programs were standard Bruker control and integration software and SHELXTL (G. M. Sheldrick).

CCDC-248593 (1) contains supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Electrochemistry: Cyclic voltammograms of dissolved monomers or oligonucleotides and of surface-bound oligonucleotides were collected on an EG&G Princeton Applied Research Potentiostat, Model 263A (using Echem software 4.11). We used a gold disc working electrode (1 mm diameter) for the solution-phase measurements and a 1-cm diameter area of a silicon surface for the surface-bound species. The counter-electrode was either a tungsten or platinum wire (1 mm diameter) in all experiments. Three different solvents were employed depending on solubility considerations: aqueous buffer, MeCN and THF. For water-soluble species we used an aqueous buffer identical to that employed for hybridisation (HEPES buffer, pH 7.5, 200 mM NaCl, 1 mM EDTA) with a saturated calomel reference electrode (SCE) and we refer to this throughout as HEPES–NaCl buffer. For reasons of solubility and for comparison with previous data, the voltammetry of some monomers was recorded in 0.1 M MeCN/NtBu₄PF₆ using a silver wire as a quasi-reference electrode (AgQRE). Some experiments on DNA-modified silicon surfaces also employed THF/1 M LiClO₄ as electrolyte. Modified silicon wafers were prepared for use as working electrodes by scratching the unpolished back side and applying a coating of In/Ga (3:1) eutectic to make ohmic contact. Electrochemistry was performed with the wafer sealed by an O-ring to the base of a 1-cm diameter Teflon cell. The electrolyte was either HEPES–NaCl buffer, or 1 M LiClO₄ in THF.

Electronic structure calculations: All calculations were performed by using the Titan program package (Wavefunction Inc., USA) running on a Dell Precision 330 workstation. Small molecule geometries were optimised in DFT by using the B3LYP functional and the LA-CVP* basis set. Geometry optimisations on the oligonucleotides were performed by using the PM3 semi-empirical Hamiltonian with initial coordinates generated by using the program B at www.scripps.edu/case/Biomer.

Oligonucleotide synthesis: An Applied Biosystems Expedite synthesiser was used for the preparation of oligonucleotides. The base-phosphoramidites were ULTRAMILD (Glen Research, VA, USA) and the standard Cap A was replaced with phenoxyacetic anhydride. Standard coupling protocols (45 s) were used with the exception of the ferrocenyl-phosphoramidite, for which the coupling time was increased to 15 min. On completion of the synthesis the column was washed thoroughly with acetonitrile and then dried with argon. Deprotection of the oligonucleotides involved treatment with MeNH₂ at a pressure of ~2 bar for 20 min. The columns were then rinsed with water to release the oligonucleotide and the washings sequentially extracted with ethyl acetate and diethyl ether.

Oligonucleotide analysis: Oligonucleotides were digested to monodeoxynucleoside constituents using snake venom phosphodiesterase and alkaline phosphatase following a literature procedure and analysed by using RP-HPLC.^[64] A Gilson system 712 controller software in association with a Gilson 811B dynamic mixer, a 802C manometric module, two Gilson Model 303 pumps and an Applied Biosystems 757 absorption detector was used for HPLC. Analytical runs were performed on a Jones APEX ODS 5µ column incubated at 30°C with an injection loop of 5 µL. The

solvent system consisted of solvent A (2.5% acetonitrile in water containing TEAA buffer pH 6.5) and solvent B (65% acetonitrile in water containing TEAA buffer pH 6.5). The 40 min digest gradient ran at 0% B for 8 min, increasing linearly to 60% B at 30 min, and decreasing back to 0% B at 40 min.

Oligonucleotides synthesised on silicon: Oriented single-crystal silicon (n-type <111>) wafers were cleaned with acetone, soaked in piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 4:1) for 10 min and then etched in deoxygenated ammonium fluoride for 15 min. The resulting hydrogen-terminated silicon wafers were then alkylated with 4,4'-dimethoxytrityl-protected ω -undecenol by refluxing in a 0.02 M solution in toluene.^[5] These wafers were used as substrates for oligonucleotide synthesis using the procedure described above by means of a custom-built Teflon cell. Dry ethyl acetate was used in the washing steps after deprotection with MeNH_2 so as to avoid potential corrosion of the surface as noted elsewhere.^[4]

Hybridisation on surfaces: Hybridisation reaction conditions involved applying the oligonucleotide solution (composition: 5–6 μL of 30 $\mu\text{mol dm}^{-3}$ oligonucleotide in 400 μL HEPES buffer pH 7.5, 200 mM NaCl, 1 mM EDTA) to the DNA-modified Si wafer, heating to 80°C and then allowing to cool to room temperature over one hour. The wafers were then rinsed with Millipore water and dried under a nitrogen flow.

Melting temperature measurements: Oligonucleotide concentrations were determined from the absorbance at 260 nm and the extinction coefficients were determined by the online calculation tools at www.basic.nwu.edu/biotools/oligocalc.html. The extinction coefficient of **1** at 260 nm is very close to that of C (see Figure 1) and therefore the same value was used as for unmodified C in this calculation. The extinction coefficient of **1-ODN** was $1.23 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and its complement **ODN*** = $1.33 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. Melting temperature curves were obtained in HEPES–NaCl buffer on a Cary 100 UV/Vis spectrophotometer. Each T_m was determined over three cycles between 12 and 90°C at $0.2^\circ\text{C min}^{-1}$.

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