DOI: 10.1002/ejoc.200700044

Reaction of Isocyanate-Functionalised Silicon Wafers with Complex Amino Compounds

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Keywords: Surface chemistry / Monolayers / Peptides / DNA / Helicene

A feasible method of grafting complex amine structures (methyl ester of glycine, Rho-Lys-Arg-NH_2, amine-terminated oligonucleotide and 7,8,11,12-tetrahydro-5-hexahelicenamine) onto a silicon wafer surface is described. The two step process comprises (1) anchoring 10-isocyanatodecyltrichlorosilane to a 1.8–2-nm thin silica layer on a single silicon wafer crystal (100) and (2) the attachment of amine substrates through a urea linkage by reacting those with isocyanate groups on the wafer surface. Both steps can be monitored by ATR FTIR showing the appearance/disappearance of the isocyanate band. Measuring the contact angles along with the AFM imaging of the surfaces indicated the formation of the monolayers.

Introduction

The covalent attachment of various molecules to silicon substrates has received much attention during the last decade, being stimulated by remarkable progress in nanoscience and nanotechnology. Indeed, silicon is considered to be a substrate of choice for such purposes as well-characterised attachment strategies have already been developed.[1] In particular, engineering silicon oxide surfaces[2] by using self-assembled monolayers to change the properties of the surface is a well-known process. There are examples in the literature of binding molecules such as fullerene,[3] chromophores,[4] flurophores[5] and biomolecules such as peptides[6] or DNA.[7]

In spite of these latest achievements, it is desired to widen the portfolio of simple and general methodologies useful for immobilising molecules on silicon substrates. Being focused on this aspect, we recently described the self-assembled monolayer of an isocyanate species on a silicon oxide surface and, moreover, we have shown the versatility of this modified surface for the covalent attachment of different simple nucleophiles as aliphatic/aromatic thiols, amines and phenols.[8] To explore the scope and limitations of our methodology, we turned to more complex molecules to be attached to the surface. Herein, we report the reaction of isocyanate-functionalised silicon wafers with biomolecules and an unnatural aromatic amino compound.

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Results and Discussion

Complex aliphatic and aromatic amines bearing the primary NH_2 group were expected to add across the isocyanate moiety to form the stable urea linkage. As suitable candidates for this purpose we chose amino acid ester **1**, peptide **2**, single-stranded DNA **3** and helicene derivative **4** (Figure 1). The selection of the nucleophiles was driven by an assumption that carrying out a new way of immobilising biomolecules such as **1**–**3** might contribute to the versatility of making biochips,[7,9] which have dramatically improved the rate of discoveries in science. A critical element in all such applications is simple attachment chemistry. We wanted to evaluate the ability of the isocyanate function to produce covalently immobilised oligonucleotides or peptides in an efficient way. Different approaches have been used for oligonucleotide immobilisation and synthetic 5′-amine-terminated oligonucleotide probes have been largely exploited.[10] In addition, immobilising large chiral molecules as **4** might be an attractive way to create chiral surfaces[11,12] that can be ultimately functionalised.

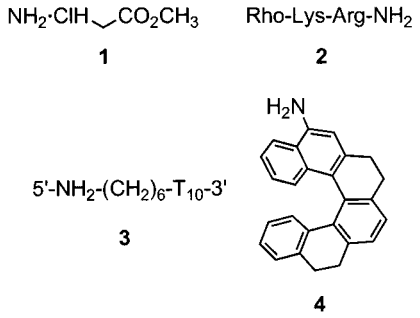
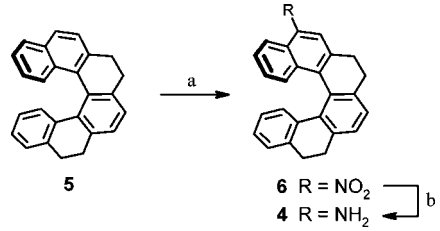


Figure 1. R-NH_2 nucleophiles subjected to the immobilisation.

As far as the nucleophiles are concerned, methyl ester of glycine **1** was commercially available and the other amino derivatives were prepared as follows. Peptide **2** was synthesised according to the procedure described elsewhere.[13] The synthesis was performed on a 0.1-mM scale by using the Fmoc/*tert*-butyl strategy on a Rink amide AMPS 1% DVB resin. After the coupling with (5)-6-carboxytetramethylrhodamine, the peptide was deprotected, cleaved from the resin and purified to give **2** in 57% yield. Homothymidinylated decamer **3** bearing a reactive primary amine group on the 5′-end was synthesised by standard phosphoramidite chemistry by using commercial 5′-amino-modifier C6.[14] The preparation of tetrahydro-5-hexaheliceneamine **4** (Scheme 1) started from tetrahydrohexahelicene **5**, which is easily accessible by utilising [2+2+2] cycloisomerisation of the aromatic triyne.[15] By treatment of **5** with nitronium tetrafluoroborate, nitro derivative **6** was isolated as the major product in good yield. The position of the nitro group was inferred from the NMR spectra and, ultimately, from the X-ray single-crystal analysis (Figure 2). The following reduction of nitro derivative **6** proceeded smoothly to obtain desired amine **4** in high yield.



Scheme 1. Synthesis of 7,8,11,12-tetrahydro-5-hexahelicenamine (**4**). Reagents and conditions: (a) NO_2BF_4 (1.1 equiv.), acetonitrile, room temp., 30 min, 56%; (b) H_2 (1 atm), 10% Pd/C, ethyl acetate, room temp., 2 h, 94%.

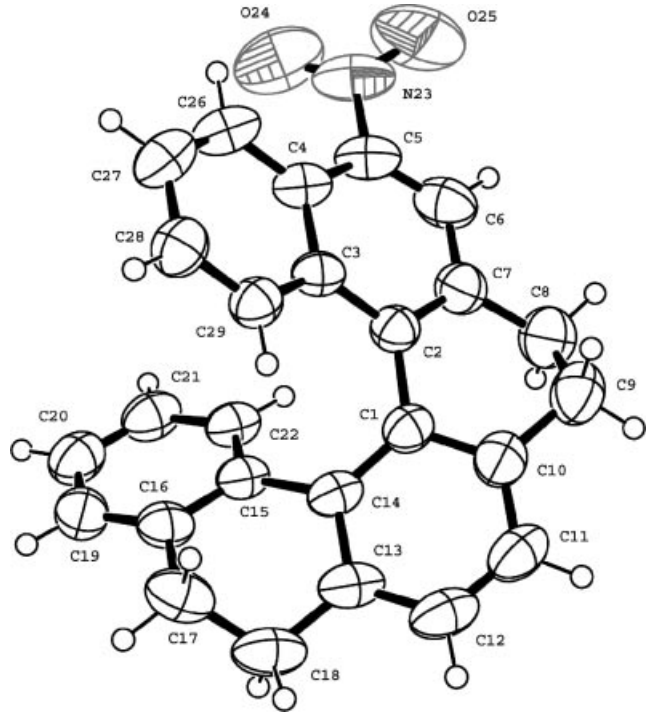
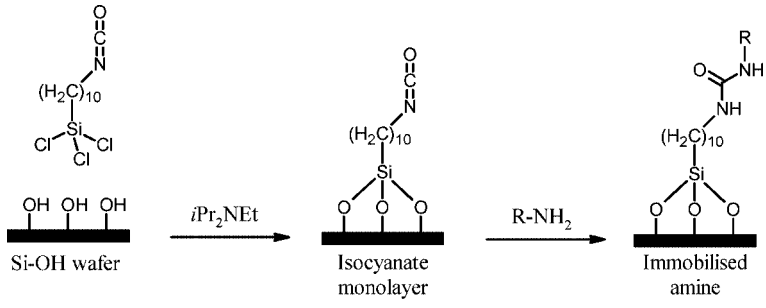


Figure 2. X-ray structure of 12-nitro-5,6,9,10-tetrahydrohexahelicene (**6**; CCDC-625162).

Having substrates **1**–**4** in our hands, we could attempt the preparation of isocyanate-terminated silicon wafers that were proposed to be utilised in the following immobilisation of the above-mentioned amino compounds. Such a surface was prepared as described previously:[8] a single silicon wafer crystal (100) with a silica layer of 1.8–2 nm thickness at the surface was treated with a solution of 10-isocyanatodecyltrichlorosilane in 1,1,2-trichloroethene in the presence of *i*Pr_2NEt at 0 °C to complete the surface modification within 50 min (Scheme 2). The reaction progress was monitored by ATR FTIR (Figure 3). The aliphatic chains were organised as monolayers as shown by the $\nu_{as}(CH_2)$ and $\nu_s(CH_2)$ bands at 2925 and 2854 cm^{-1}, respectively.

In order to graft the amine substrates, we used the following procedures. The methyl ester of glycine **1** was dissolved in 1,1,2-trichloroethene (10^{-1} M) and treated with the



Scheme 2. Preparation of isocyanate-terminated silicon wafers and the immobilisation of amines.

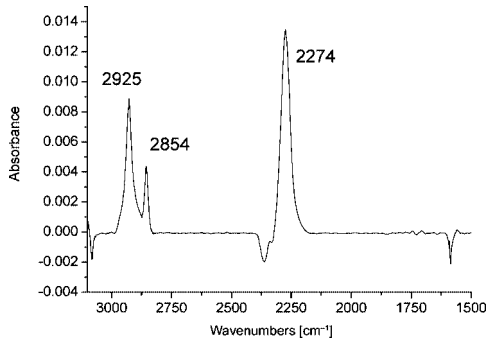


Figure 3. ATR FTIR of the silicon wafers functionalised by isocyanate.

isocyanate-functionalised silicon wafer in the presence of iPr$_2$NEt at 0 °C. We observed the immediate disappearance of the isocyanate band at 2274 cm^{-1} and the appearance of the ester band at 1745 cm^{-1} (Figure 4).[16] The amide band was detected at 1633 cm^{-1}. A little amount of iPr$_2$EtN was physisorbed after washing (band at 2976 cm^{-1}). The contact angle measurement was 75°, which is in agreement with the formation of a methyl ester monolayer.[17] Ellipsometry indicated a thickness of 14 Å, which corresponds to a monolayer with tilting of the chains due to gauche conformations (the thickness of 20 Å could be expected with fully extended chains perpendicular to the surface).

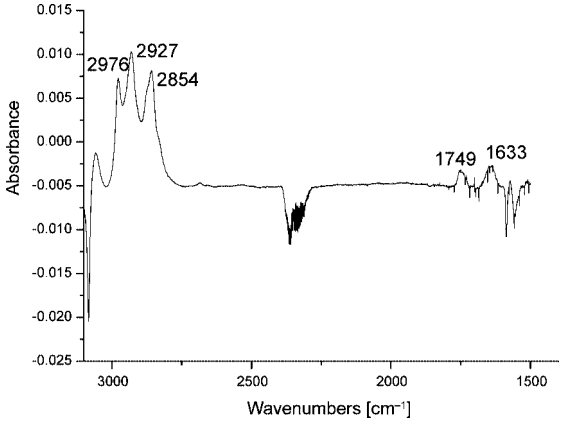


Figure 4. The reaction of amine **1** with the isocyanate-modified silicon wafer (ATR FTIR spectrum).

To graft more complex structures, peptide **2** was dissolved in DMF (7×10^{-1} μM) with a catalytic amount of EtOH and then treated with the isocyanate-functionalised silicon wafer with iPr$_2$NEt at 0 °C. The reaction was thoroughly analysed by ATR FTIR to show the disappearance of the isocyanate band after 1 h. The contact angle was 71°, indicating a rather hydrophilic surface. Ellipsometry showed a thickness of 21 Å, which indicated the formation of a monolayer, with tilting of the aliphatic chains due to gauche conformations in the monolayer. Scanning the wafer surface with AFM, a grain-like structure was revealed (Figure 5), which is typical for a surface fully covered by peptide.[18] These results were in accord with the proposed attachment of peptide **2** by its lysine side chain to the functionalised wafer.

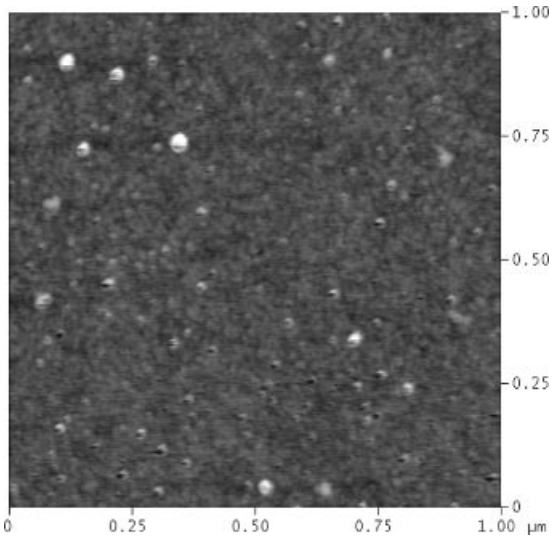


Figure 5. Tapping mode AFM of peptide **2**-modified silicon surface (roughness analysis). Rms = 0.335 nm.

To check the viability of immobilising nucleic acid fragments by using this method, single-stranded DNA **3** was dissolved in DMF (2.5×10^{-1} M) with a catalytic amount of EtOH and then treated with the isocyanate-terminated silicon wafer at 0 °C. After 1 h, the total disappearance of the isocyanate band was observed. Ellipsometry indicated a thickness of 18 Å, which showed the formation of a monolayer with tilting and the DNA strand standing flat on the surface. Afterwards, the 76° value of the contact angle was in agreement with a rather hydrophilic surface. The observed grain-like structure of the wafer surface that was visualised with AFM (Figure 6) indicated the DNA strand attachment.[19]

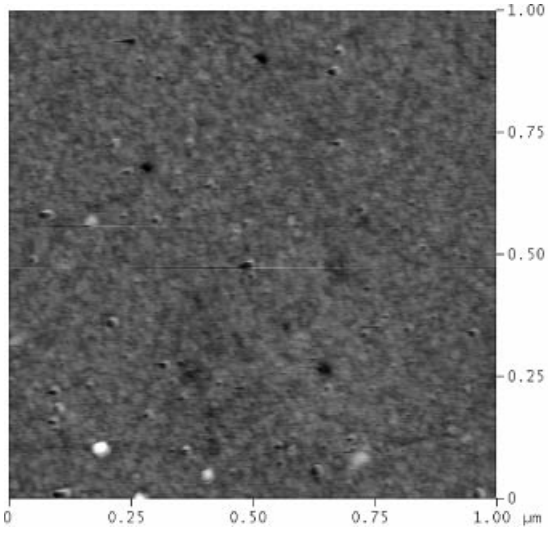


Figure 6. Tapping mode AFM of DNA **3**-modified silicon surface (roughness analysis). Rms = 0.327 nm.

Finally, we attempted grafting racemic helicene derivative **4**, which represents a completely artificial structure. Helical amine **4** was dissolved in 1,1,2-trichloroethene (5.10^{-4} M) and the isocyanate-functionalised silicon wafer was treated

with this solution at 0 °C. The reaction went to the completion overnight as evidenced by the disappearance of the isocyanate band. The proposed formation of an aromatic system monolayer was in a good agreement with the 91° value of the contact angle recorded.[20] AFM measurement of the wafer surface pictured a grain-like structure (Figure 7). Ellipsometry revealed a thickness of 19 Å, which is in agreement with the formation of a helicene monolayer with tilting of the aliphatic chains.

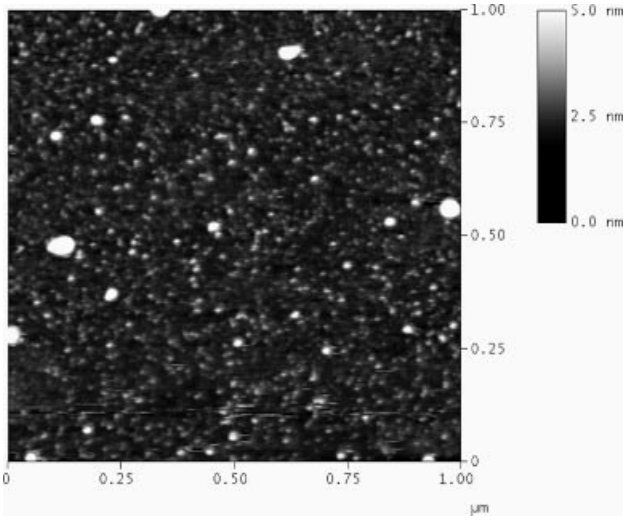


Figure 7. Tapping mode AFM of helicene derivative **4**-modified silicon surface (roughness analysis).

Conclusions

We demonstrated a feasible method for grafting complex amine structures onto a silicon wafer surface. This is a two-step process comprising (1) surface modification with 10-isocyanatodecyltrichlorosilane and (2) attachment of amine substrates through a urea linkage by treating those with isocyanate groups on the wafer surface. Both steps can be monitored by ATR FTIR showing the appearance/disappearance of the isocyanate band. Measuring the contact angles along with the AFM images of the surfaces indicate independently the formation of the proposed monolayers.

Experimental Section

General: ^{1}H NMR spectra were measured at 200.0, 499.8 or 500.13 MHz, ^{13}C NMR spectra at 125.7 MHz, in $CDCl_3$ with TMS as an internal standard. Chemical shifts are given in δ-scale, coupling constants J are given in Hz. HMBC experiments were set up for J_{C-H} = 5 Hz. For correct assignment of both ^{1}H and ^{13}C NMR spectra of key compounds, COSY, ROESY, HMQC, HMBC and CIGAR-HMBC experiments were performed. For all the other compounds, the general semiempirical equations were used for the chemical shift assignment. IR spectra were measured in $CHCl_3$ or in KBr pellets. ATR infrared spectra measured in situ were recorded with an FTIR Perkin–Elmer 2000 spectrometer equipped with narrow band liquid nitrogen cooled MCT detector and a thermoregulated ATR flow cell. The sample compartment was purged with dry air. All the spectra were recorded at a resolution of 1 cm^{-1}, and 128 scans were accumulated. The size of the ATR crystal was 70 × 10 × 1 mm and 35 reflections were used. EIMS spectra were determined at an ionising voltage of 70 eV, *m*/*z* values are given along with their relative intensities (%). FABMS spectra were measured by using a thioglycerol/glycerol (3:1) matrix, *m*/*z* values are given. HRMS spectra were obtained by the EI or FAB modes. Tetrahydrohexahelicene **5** was prepared according to the literature.[15] Commercially available reagent grade materials were used as received. Acetonitrile and diisopropylethylamine were distilled from calcium hydride under an atmosphere of argon, dimethylformamide was distilled from calcium hydride under vacuum, trichloroethylene was freshly distilled prior to use and ethyl acetate (p.a. grade) was used as received. TLC was performed on Silica gel 60 F_{254}-coated aluminium sheets (Merck) and spots were detected with a solution of $Ce(SO_4)_2 \cdot 4H_2O$ (1%) and $H_3P(Mo_3O_{10})_4$ (2%) in sulfuric acid (10%). Flash chromatography was performed on Biotage KP-Sil Silica cartridges (0.040–0.063 mm) used in an Sp1 HPFC system (Biotage, Inc.). HPLC water (Acros Organics) was used to rinse the Si(100) substrates.

CCDC-625162 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

12-Nitro-5,6,9,10-tetrahydrohexahelicene (6): NO_2BF_4 (26 mg, 0.223 mmol, 1.1 equiv.) in dry acetonitrile (2 mL) was added to racemic **5** (70 mg, 0.211 mmol) in dry acetonitrile (7 mL), and the solution was stirred under an atmosphere of argon at room temp. for 30 min. The solvent was removed in vacuo, and the residue was chromatographed on silica gel (petroleum ether/ether 99:1 to 90:10) to obtain nitro derivative **6** (45 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.67–2.78 (m, 2 H), 2.88–3.10 (m, 6 H), 6.22 (ddt, J = 7.9, 1.4, 0.6, 0.6 Hz, 1 H), 6.39 (dddd, J = 7.9, 7.3, 1.4, 0.8 Hz, 1 H), 6.84 (dt, J = 7.5, 7.5, 1.3 Hz, 1 H), 6.95 (ddd, J = 8.8, 6.8, 1.2 Hz, 1 H), 7.17 (dtt, J = 7.4, 1.3, 1.3, 0.5, 0.5 Hz, 1 H), 7.30 (s, 2 H), 7.32 (ddd, J = 8.8, 6.8, 1.3 Hz, 1 H), 7.60 (br. ddd, J = 8.7, 1.3, 0.7 Hz, 1 H), 8.29 (s, 1 H), 8.39 (br. ddd, J = 8.8, 1.3, 0.6 Hz, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 29.73 (t), 29.80 (t), 30.24 (t), 30.45 (t), 122.47 (d), 124.08 (d), 124.79 (s), 125.51 (d), 125.90 (d), 126.45 (d), 126.79 (2 × d), 127.33 (d), 127.61 (d), 127.97 (d), 128.43 (d), 129.51 (s), 130.35 (s), 134.45 (s), 135.44 (s), 136.46 (s), 137.96 (s), 138.53 (s), 139.28 (s), 141.00 (s), 144.94 (s) ppm. IR: $\tilde{\nu}$ = 3054 (w), 1620 (vw), 1603 (w), 1590 (vw), 1556 (m), 1517 (vs), 1488 (w), 1419 (w), 1552 (m), 1334 (s), 1325 [s (sh)], 1254 (w), 1165 (w), 1115 (w), 1023 (w), 898 (w), 879 (w), 825 (m), 816 (m), 690 (w), 588 (w), 494 (w), 466 (w) cm^{-1}. EIMS: *m*/*z* (%) = 377 (100) $[M]^{+\cdot}$, 347 (32), 329 (7), 315 (13), 256 (6), 97 (15), 83 (18), 69 (29), 57 (40). HREIMS: calcd. for $C_{26}H_{19}NO_2$ 377.1416; found 377.1426.

7,8,11,12-Tetrahydro-5-hexahelicenamine (4): Nitro derivative **6** (30 mg, 0.080 mmol) in ethyl acetate (6 mL) was hydrogenated over 10% Pd on charcoal (5 mg) under the atmospheric pressure of hydrogen. The mixture was stirred at room temp. for 2 h, filtered through a paper filter and the solvent was removed in vacuo to receive pure amine **4** (26 mg, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.63–2.97 (m, 8 H), 6.37 (dddd, J = 7.9, 7.3, 1.3, 0.9 Hz, 1 H), 6.48 (dd, J = 7.9, 1.3 Hz, 1 H), 6.81 (s, 1 H), 6.81 (ddd, J = 7.5, 7.3, 1.3 Hz, 1 H), 6.84 (ddd, J = 8.6, 6.7, 1.3 Hz, 1 H), 7.11 (ddd, J = 8.4, 6.7, 1.3 Hz, 1 H), 7.15 (br. dt, J = 7.5, 0.8, 0.8 Hz, 1 H), 7.15 (dd, J = 7.4, 0.8 Hz, 1 H), 7.22 (dd, J = 7.4, 1.0 Hz, 1 H), 7.48 (ddt, J = 8.6, 1.3, 0.8, 0.8 Hz, 1 H), 7.64 (ddt, J = 8.4, 1.3, 0.7, 0.7 Hz, 1 H) ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 29.98

(2 × t), 30.50 (t), 31.25 (t), 110.12 (d), 120.02 (d), 122.75 (s), 123.32 (d), 123.56 (s), 124.71 (d), 125.02 (d), 125.17 (d), 125.89 (d), 125.95 (d), 126.58 (d), 126.87 (d), 127.78 (d), 130.18 (s), 131.37 (s), 133.30 (s), 135.59 (s), 137.72 (s), 137.94 (s), 139.04 (s), 139.91 (s), 141.49 (s) ppm. IR (KBr): $\tilde{\nu}$ = 3462 (m), 3374 (m), 3054 (w), 3016 (w), 2930 (s), 2895 (m), 2833 (m), 1619 (s), 1583 (m), 1561 (m), 1515 (w), 1487 (w), 1458 (m), 1399 (s), 1366 (m), 1329 (w), 1277 (m), 1266 (m), 1190 (w), 848 (m), 824 (m), 793 (w), 781 (m), 742 (s), 708 (m), 619 (m), 576 (m), 539 (w) cm^{-1}. FABMS: *m/z* (%) = 348 $[M + 1]^+$, 347 $[M]^+$. HRFABMS: calcd. for $C_{26}H_{21}N$ 347.1674; found 347.1683.

X-ray Crystallography of Nitro Derivative 6: X-ray crystallographic analysis of a single crystal of **6** (yellow, 0.13 × 0.19 × 0.26 mm) was performed with an Xcalibur X-ray diffractometer with Cu-K_α (λ = 1.54180 Å), data collected at 295 K. The structure was solved by direct methods with SHELXS 86[21] and refined by full-matrix least-squares on *F* with CRYSTALS.[22] All H atoms were located in a difference map, and they were repositioned geometrically and then refined with riding constraints. Crystal data: $C_{26}H_{19}NO_2$; triclinic; space group $P\bar{1}$; a = 8.0168(10), b = 9.2014(12), c = 26.3482(18) Å; α = 79.947(8), β = 89.986(8), γ = 86.899(10)°; V = 1910.9(4) $Å^3$; Z = 4; M = 377.42; 27535 reflections measured; 7608 independent reflections. Final R = 0.0790, wR = 0.0347 for 4006 reflections with $I > 1.96\sigma(I)$ and 523 parameters.

Nucleophiles Grafted on Si(100) Surfaces: (a) Preparation of Oxidised Silicon Si(100) Surface: First, the native oxide layer was removed by immersion in aqueous HF solution (40%) until total dewetting of the surface (about 10 s). The substrates were cleaned by rinsing with water. The substrates were then exposed in a home-built UV-ozone chamber. This technique is well-known to eliminate all organic impurities from a surface but was also used to oxidise silicon and obtain a hydrated flat silica surface free from organic pollution. The wafers were placed at a maximum distance of 5 mm from a two-wavelength low-pressure mercury lamp (λ = 185 and 254 nm) under an O_2 stream. After 30 min of exposition, a hydrophilic silica surface (contact angle $\theta < 10°$) was obtained. Its thickness, measured by ellipsometry, was about 1.8–2.0 nm and its roughness, measured by tapping mode atomic force microscopy, was about 0.15 nm.

(b) Grafting of 10-Isocyanatodecyltrichlorosilane on the Oxidised Silicon Surface: The oxidised silicon wafer was placed into a Schlenk tube under a nitrogen atmosphere. The grafting solution containing 10-isocyanatodecyltrichlorosilane (10^{-2} M) and diisopropylethylamine (10^{-1} M) in trichloroethylene was introduced into the tube. The wafer was treated with the solution at 0 °C for 45 min under a nitrogen atmosphere without stirring. Then, the solution was removed, and the wafer was washed with trichloroethylene, THF and trichloroethylene.

(c) Reaction of the Immobilised Isocyanate with Glycine Methyl Ester (1): Immediately after washing the wafer surface in the Schlenk tube, a solution containing glycine methyl ester **1** (10^{-1} M) in trichloroethylene and diisopropylethylamine (8×10^{-1} M) was introduced. The wafer was treated with the solution at 0 °C for 2 h under a nitrogen atmosphere. Then, the solution was removed, and the wafer was rinsed with methanol (5 min), THF (5 min), trichloroethylene (5 min) and dried in a stream of nitrogen.

(d) Reaction of the Immobilised Isocyanate with Rho-Lys-Arg-NH_2 (2): Immediately after washing the wafer surface in the Schlenk tube, a solution containing rhodaminated dipeptide **2** (7×10^{-1} μM) in DMF (2 mL) and EtOH (0.1 mL) was introduced followed by diisopropylethylamine (7 μM). The wafer was treated with the solution at 0 °C for 2 h under a nitrogen atmosphere. Then, the liquid phase was removed, and the wafer was rinsed with methanol (5 min), THF (5 min), trichloroethylene (5 min) and dried in a stream of nitrogen.

(e) Reaction of the Immobilised Isocyanate with 5′ NH_2-$(CH_2)_6$-T_{10}-3′ (3): Immediately after washing the surface in the Schlenk tube, a solution containing homothymidinylated decamer **3** (2.5×10^{-1} M) in DMF (2 mL) and EtOH (0.1 mL) was introduced. The wafer was treated with the solution at 0 °C for 2 h under a nitrogen atmosphere. Then the solution was removed, and the wafer was rinsed with methanol (5 min), THF (5 min), trichloroethylene (5 min) and dried in a stream of nitrogen.

(f) Reaction of the Immobilised Isocyanate with 7,8,11,12-Tetrahydro-5-hexahelicenamine (4): Immediately after washing the wafer surface in the Schlenk flask, a solution containing 7,8,11,12-tetrahydro-5-hexahelicenamine **4** (5×10^{-1} mM) in trichloroethylene was introduced. The wafer was treated with the solution at 0 °C overnight under a nitrogen atmosphere. Then, the solution was removed, and the wafer was rinsed with methanol (5 min), THF (5 min), trichloroethylene (5 min) and dried in a stream of nitrogen.

Supporting Information (see footnote on the first page of this article): ATR FTIR spectra of DNA **3**, peptide **2** and helicene **4**, which were treated with the isocyanate monolayer.

Acknowledgments

This research was supported within the Programme Barrande by the Ministry of Education of the Czech Republic and the French Ministry of Foreign Affairs (Project 2005–06–041–1), by the Ministry of Education of the Czech Republic (Centre for Biomolecules and Complex Molecular Systems, Project LC512) and by the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic (this work is part of the Research project Z4 055 0506). We thank Dr. Michel Ramonda for AFM measurements.

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Received: January 19, 2007
Published Online: June 25, 2007

