Pentafluorinated Probes for the X-ray Photoelectron Spectroscopic Study of Immobilized Bifunctional Silanes

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Bifunctional silanes constitute valuable linking agents for attachment of biomolecules at high levels of surface population density through the formation of thioether or disulfide bonds. Three such compounds, 1-((trifluoroacetyl)thio)-11-(trichlorosilyl)undecane, 1-bromo-11-(trichlorosilyl)undecane, and 1-((bromoacetyl)oxy)-11-(trichlorosilyl)undecane, are discussed in terms of their surface chemistry on silicon wafers. To examine the electrophilic and nucleophilic generation of sulfur-containing linkages, three new probes, *N*-(pentafluorophenyl)iodoacetohydrazide, *N*-(pentafluorophenyl)-3-(2-pyridylthio)proprionohydrazide, and *N*-(pentafluorophenyl)mercaptoacetohydrazide, are introduced with respect to their reactions with silanized surfaces (studied by X-ray photoelectron spectroscopy). Thiol-functionalized surfaces obtained by silanization act as nucleophiles toward the probes. In air, low yields of conjugation are exhibited which are attributed to the unavailability of thiol groups because of intramolecular disulfide group formation instigated by oxygen or by disulfide exchange with the proprionyl probe. The behavior of electrophilic silanized surfaces toward the mercaptoacetyl-containing probe is governed by the nature of the leaving group and by steric factors.

A key element of the fabrication of genosensors is the attachment of a single strand of oligonucleotide or DNA to the surface of a particular transducer. Included in the portfolio of methods available for this purpose are chemisorption, cross-linking and encapsulation, complexation (avidin−biotin chemistry), direct oligonucleotide−thiol (to gold) binding, and oligonucleotide synthetic protocols based on porous silica−silane chemistry.[1−12] Although limited success can be achieved using these approaches,

a number of detrimental factors are evident, including nucleic acid wash-off, poor levels of surface population, removal at typical annealing temperatures, and multipoint attachment leading to the highly disadvantageous lack of access to the correct nucleic acid tertiary structure. Accordingly, we are developing new strategies for oligonucleotide immobilization.

To achieve the generation of a high oligonucleotide population on surfaces, we have synthesized new bifunctional silanes which are capable of the high-density thiol functionalization of hydroxylated substrate surfaces. These molecules, ((1-trifluoroacetyl)oxy)-11-(trichlorosilyl)undecane (TTU) and 1-bromo-11-(trichlorosilyl)undecane (BTU), after surface silanization reactions, can be manipulated chemically to yield a layer of thiol functionalities. The significant advantages of the thiol group lie in its ability to participate in an S_N2-type thiol−disulfide exchange process or the formation of an intractable thioether bond. Accordingly, a surface population of thiol groups offers a number of pathways to oligonucleotide and DNA immobilization through the appropriate facile derivatization of the nucleic acid. At this point, it is necessary to emphasize that the standard linking agent, (mercaptopropyl)-trimethoxysilane (MPS), produces poorly formed multilayer structures that provide limited access to thiol group chemistry.[13]

In the present paper, we describe the synthesis and characterization of new probes for the XPS examination of sulfur-based conjugation reactions on surfaces. We chose organosilane adhesion agents with an alkyl chain length of ≥ 8 carbons because they form thin, well-ordered films on a wide variety of hydroxyl-containing substrates.[14−23] With respect to interfacial reactions,

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the deprotected TTU (diluted with octyltrichlorosilane (C8)) surface is examined in terms of its nucleophilic action on all three probes. Additionally, we introduce a new bifunctional silane, 1-((bromoacetyl)oxy)-11-(trichlorosilyl)undecane (BATU),[18] which, like TTU[24] and BTU,[19] is capable of thiol functionalization when employed in conjunction with surface-modifying reactions. However, in the present work, we restrict our study to an evaluation of the surface behavior of both BTU and BATU with regard to their capabilities of functioning as electrophiles toward the mercaptoacetyl probe.

EXPERIMENTAL SECTION

Reagents and Materials. ω-Undecenyl alcohol, 98%, bromoacetic acid, 99+%, hydrogen hexachloroplatinate(IV) hydrate, 99.995%, octyltrichlorosilane (C8), 99%, potassium thioacetate, 98%, iodoacetic acid, 98%, 1,3-dicyclohexylcarbodiimide (DCC), 99%, 4-(dimethylamino)pyridine (DMAP), 99%, 3-(2-pyridyldithio)propionic acid N-hydroxysuccinimide ester, 98%, (pentafluorophenyl)-hydrazine, 97%, 2,2′-dipyridyl disulfide, silica gel (Merck, grade 9385, 230−400 mesh, 60 Å), and 2,6-lutidine were obtained from Aldrich and were used as received. Hydroxylamine hydrochloride, dimethylformamide, methanol, anhydrous magnesium sulfate, hexanes, diethyl ether, tetrahydrofuran, methanol, and hydrochloric acid were purchased from BDH and were used without further purification. Dichloromethane and acetonitrile were also purchased from BDH and were distilled over phosphorus pentoxide before use.

Silicon wafers obtained from International Wafer Service were supplied approximately 0.4 mm thick and were polished on one side to a mirror finish. They were cut to a size of approximately 1×1 cm using a diamond-tipped pencil.

Instrumentation. NMR spectra are reported in units of δ and were recorded either on a Varian Gemini 200 spectrometer using a ^{1}H−^{13}C switchable probe or on a Varian VXR 400S spectrometer (^{1}H, ^{13}C, ^{19}F, ^{29}Si) using a 5 mm switchable probe. In the case of ^{29}Si, either inverse-gated decoupling or DEPT was used. The samples were dissolved in CDCl$_3$ which contained 0.03% TMS. Both ^{1}H and ^{29}Si NMR spectra were referenced to TMS at 0.00 ppm, while ^{13}C NMR spectra were referenced to the center of the CDCl$_3$ triplet at 77.00 ppm. ^{19}F NMR spectra were calibrated using an external CFCl$_3$ standard (0.00 ppm).

Elemental analysis was performed by Canadian Microanalytical Service Ltd. (Delta, BC, Canada). The trichlorosilanes were handled in an inert atmosphere during transport and analysis. All elements except oxygen were determined for the TTU sample.

X-ray photoelectron spectra were recorded on a Leybold MAX-200 X-ray photoelectron spectrometer using an unmonochromatized Mg Kα source run at 15 kV and 20 mA. The energy scale of the spectrometer was calibrated to the Ag 3d$_{5/2}$ and Cu 2p$_{3/2}$ peaks at 368.3 and 932.7 eV, respectively. The binding energy scale was calibrated to 286 eV for the main C(1s) feature. For all samples, a survey run (pass energy = 192 eV, 0−1000 eV on the binding energy scale, X-ray analysis area size 4×7 mm, analyzed at a 90° angle relative to the electron detector) was performed, and a higher resolution series of experiments were performed. Angle-resolved XPS spectra were obtained using a 2×4 mm analysis area at various angles relative to the surface normal. Satellite

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N-Pentafluorophenyl-N'-3-(2-pyridyldithio)-propionyl hydrazide (PD F5)
$C_{14} H_{10} F_5 N_3 O S_2$ MW = 395.36

N-Pentafluorophenyl-N'-iodoacetyl hydrazide (Iac F5)
$C_8 H_4 F_5 N_2 O I$ MW = 366.02

N-Pentafluorophenyl-N'-mercaptoacetyl hydrazide (Mac F5)
$C_8 H_5 F_5 N_2 O S$ MW = 272.20

1-Bromo-11-(trichlorosilyl)-undecane
$C_{11} H_{22} Br Cl_3 Si$ MW = 368.62

1-(Thiotrifluoroactetato)-11-(trichlorosilyl)-undecane
$C_{13} H_{22} Cl_3 F_3 O S Si$ MW = 416.75

1-(Bromoacetato)-11-(trichlorosilyl)-undecane
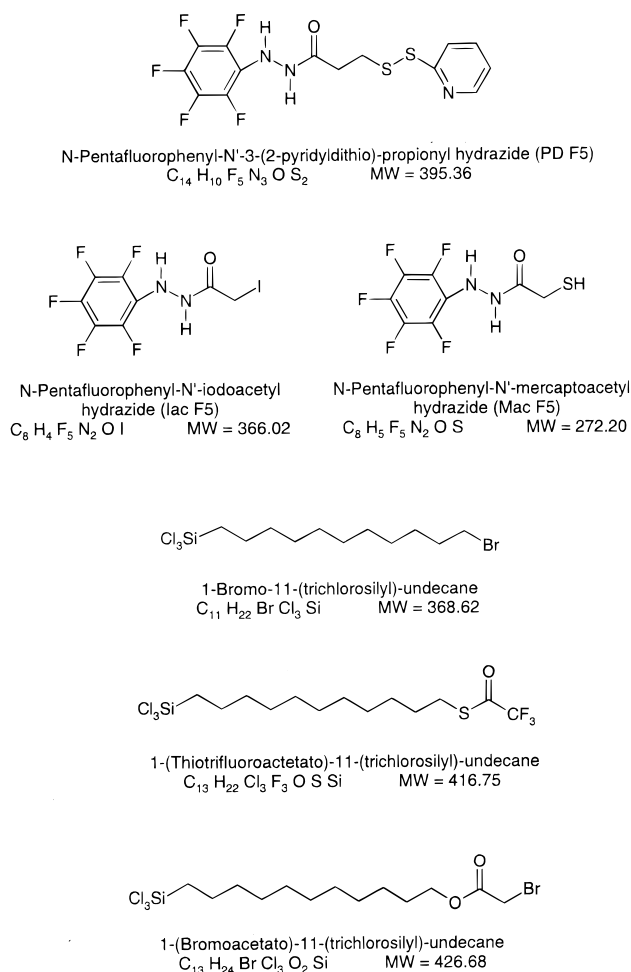$C_{13} H_{24} Br Cl_3 O_2 Si$ MW = 426.68

Figure 1. Molecular structures of three bifunctional silanes and three pentafluorinated probes.

subtraction and normalization were performed with software obtained from the manufacturer. Quantitation of the spectra were performed using empirically derived sensitivity factors (obtained from the manufacturer) (for a Mg anode); the sensitivity factors were C(1s) = 0.34, O(1s) = 0.78, F(1s) = 1.00, and Si(2p) = 0.4. Peak fitting and data analysis were performed using ESCA Tools software.

Finally, the contribution of carbon occurring as a result of adventitious contamination was assessed carefully and compared with values obtained for silanized layers. On no occasion was a C(1s) signal from a blank, control sample greater than 15% of the value for a silanized film.

Mass spectrometry was performed on a VG 70-250S (double-focusing) mass spectrometer. The sample was subjected to electron ionization at 70 eV and an accelerating voltage of 8 keV. The source was set at 250 °C and a pressure of 10^{-6} mbar. Perfluorokerosene was introduced into the spectrometer via a separate, continuous-introduction system, and the CF$_3^+$ ion (mass 68.9952) was used as a reference. Under these conditions, the mass spectrometer had a resolution of about 1200 ($m/\Delta m$) at a 10% valley.

Synthesis. The chemical structures of the various silanes and probes discussed in this work are depicted in Figure 1. The two silanes, TTU and BTU, were prepared as described elsewhere.[24]

The synthetic procedures employed for the preparation of the three probes and BATU are provided as Supporting Information. Analytical data for each compound are as follows:

N-(Pentafluorophenyl)-3-(2-pyridyldithio)proprionohy-drazide (PD F5). ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.8 (1H, s), 8.55 (1H, m), 7.75 (2H, m), 7.18 (1H, m), 6.35 (1H, s), 3.09 (2H, t), 2.70 (2H, t). ^{13}C NMR (400 MHz, CDCl$_3$): δ 171.46, 158.65, 149.51, 140.34, 139.15, 137.82, 137.21, 136.53, 135.23, 123.48, 121.54, 121.32, 34.99, 33.72. LRMS, m/z: calcd for C$_{14}$H$_{10}$F$_5$N$_3$-OS$_2$ (M)$^+$, 395; found, 395. Anal. Calcd: C, 42.53; H, 2.55; F, 24.03; N, 10.62; S, 16.22. Found: C, 42.22; H, 2.46; F, 22.16; N, 10.33; S, 16.25.

N-(Pentafluorophenyl)iodoacetohydrazide (Iac F5). ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.94 (1H, s), 6.24 (1H, s), 3.68 (2H, s). ^{13}C NMR (400 MHz, CDCl$_3$): δ 167.15, 140.35, 139.03, 137.94, 136.52, 135.63, 122.43, −5.44. LRMS, m/z: calcd for C$_8$H$_4$F$_5$N$_2$OI (M)$^+$, 366; found, 366. Anal. Calcd: C, 26.25; H, 1.10; F, 25.96; N, 7.65; I, 34.67. Found: C, 26.72; H, 1.17; F, 22.52; N, 7.57; I, 33.31.

N-(Pentafluorophenyl)mercaptoacetohydrazide (Mac F5). ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.8 (1H, s), 8.35 (1H, s), 6.24 (1H, s), 3.25 (1H, d, J = 9.2 Hz), 2.0 (2H, t). ^{13}C NMR (400 MHz, CDCl$_3$): δ 169.08, 140.21, 139.03, 137.83, 136.55, 135.49, 122.76, 26.45. LRMS, m/z: calcd for C$_8$H$_5$F$_5$N$_2$OS (M)$^+$, 272; found, 272. Anal. Calcd: C, 35.30; H, 1.85; F, 34.90; N, 10.28; S, 11.78. Found: C, 35.50; H, 2.22; F, 29.42; N, 10.08; S, 13.14.

1-((Bromoacetyl)oxy)-11-(trichlorosilyl)undecane. ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.18 (2H, t), 3.82 (2H, s), 1.20−1.80 (20H, m). ^{13}C NMR (400 MHz, CDCl$_3$): δ 167.17, 66.39, 33.74, 31.80, 31.25, 29.37, 29.32, 29.11, 29.03, 28.86, 28.36, 25.92, 25.69. ^{29}Si NMR (400 MHz, CDCl$_3$): δ 13.32. Anal. Calcd: C, 36.59; H, 5.67; Br, 18.73; Cl, 24.93; Si, 6.58. Found: C, 40.36; H, 6.36; Br, 37.35; Cl, 19.03; Si, <0.2.

Silanization. The silicon wafers were first gently washed with detergent, and then rinsed copiously with distilled water before drying in air. They were then washed with chloroform and dried with a stream of N$_2$ before sonication in 30% hydrogen peroxide for 30 min. After being rinsed copiously with water, the wafers were dried in an oven at 120 °C for 30 min. The wafers were stored in a humidity chamber which contained water saturated with Mg-(NO$_3$)$_2$ overnight. The hydrated silicon substrates were then rapidly transferred to test tubes that had been previously silanized with octadecyltrichlorosilane. The test tubes were stoppered, and the substrates were silanized in a drybox by immersion in a 1 × 10^{-3} M solution of 50% silane/50% C8 in dry toluene (50% on a molar basis). The samples were rinsed with both toluene and chloroform before drying with a stream of N$_2$. Following this procedure, wafers, together with those outlined below, were always stored under a double nitrogen gas seal to minimize adventitious contamination from carbon.

Deprotection of the Thiol Group of TTU. The trifluoroacetyl functionality was removed in air and under O$_2$-free conditions. In the former case, the TTU wafers were treated with 2 mL of 0.5 M NH$_2$OH in water (pH 8.5) for 2 h at room temperature, rinsed with a copious amount of deionized water and with methanol, and then blown dry with N$_2$ before storage in clean screw-capped vials. For experiments involving O$_2$-free conditions, the TTU wafers were placed in test tubes fitted with septa and purged with N$_2$. A 0.5 M NH$_2$OH solution was purged with He for 15 min, and 2 mL of the

solution was added to each test tube containing the wafer. The wafers were soaked in a hydroxylamine solution for 2 h at room temperature while under N$_2$, with the solution being removed by syringe. The wafers were rinsed with five 2 mL aliquots of He-sparged deionized water and then with three 2 mL aliquots of He-sparged methanol. All operations were performed under conditions such that the wafers were never exposed to the outside air. Substrates were left inside test tubes under nitrogen prior to probe reactions.

Probe Reactions with Silane-Modified Surfaces. The reactions were performed within 2 h of silanization of surfaces. Wafers with deprotected TTU in place were generally immersed in 10^{-3} M solutions of the probes in 80% DMF which contained 20% 2,6-lutidine (overnight at room temperature). For cases where O$_2$-free conditions were required, all experiments were performed under N$_2$ and liquids were sparged with He. After the various reactions, the substrates were rinsed with five 2 mL aliquots of DMF and then three 2 mL volumes of methanol before being dried in a stream of nitrogen. In certain experiments, sequential probe reactions were performed such as treatment with 2,2′-dipyridyl disulfide followed by exposure to Mac F5. Similar protocols involved Iac F5 followed by Mac F5.

The conjugation of BTU and BATU surface-modified wafers with Mac F5 was effected using the same produce specified above for the TTU experiments.

RESULTS AND DISCUSSION

Silanization Strategy and Probe Design. Before describing a detailed study of the reactions of the three probes with the silanized surfaces, it is necessary to outline aspects of the silanization protocol and probe the design employed in the present work. With regard to the method chosen for silanization of substrate surfaces, the silicon wafers were subjected to an atmosphere of controlled humidity prior to the reactions conducted in dry toluene.[25] The reason for adaptation of this technique lies in the mechanism that is involved when silanizing reactions occur at substrate surfaces. The quality and density of silane films formed on surfaces are governed by the extraction of water from the interface into bulk solution by the anhydrous reaction medium.[26] Accordingly, we have incorporated deliberate moisture introduction into the silanization procedure to optimize the generation of films of the various silanizing reagents. A second point concerns the dilution of the reacting bifunctional silanes, BTU, TTU, and BATU (termed active silanes here), with octyltrichlorosilane to a level of 50% of the total silane. We have found that this dilution level represents a practical value in terms of the minimization of thiol group cross-linking reactions and immobilization of derivatized oligonucleotide on the substrate surface.

The rationale behind the design of the pentafluorinated molecular probes in the present study was based on the following criteria. The probe must contain the necessary reactive functionalties for conjugation to the substrate surface, react in a highly reproducible fashion, and contain atoms that deliver sensitive detection by XPS,[27] the surface analytical technique of choice in this work. With regard to the latter, the pentafluorophenyl group

(25) Canadian Patent Application 2,213,087, 1997; U.S. Patent Application 8,951,448, 1997 (pending).
(26) McGovern, M. E.; Kallury, K. M. R.; Thompson, M. *Langmuir* **1994**, *10*, 3607.

was chosen since this functionality is chemically stable and provides a sensitive signal in XPS. This group is found in (pentafluorophenyl)hydrazine, which can be reacted with a variety of carboxylic acid derivatives to yield hydrazides that can be readily purified by recrystallization. The terminal nitrogen of the hydrazine moiety appears to be relatively insulated from the electron-withdrawing pentafluorophenyl ring by its nitrogen neighbor and reacts as a nucleophile without difficulty. Each pentafluorophenyl-labeled probe contains five XPS-equivalent fluorines, which are found at 687 eV in the XPS spectrum and are different from those of the trifluoromethyl group, which are found at 689 eV. The two different fluorine chemical shifts can be used to discriminate the pentafluorophenyl-labeled probes from the TFA-protecting group of TTU if both are present on the same surface.
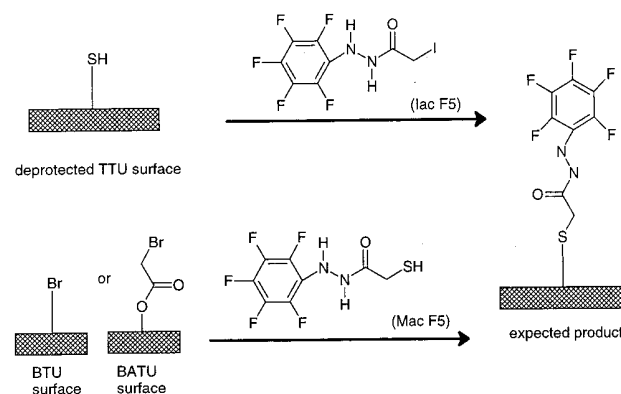
Two main classes of nucleophilic reactions involving deprotected TTU on surfaces can be performed using the pentafluorophenyl-labeled probes. First, the thiol groups on the substrate surface can act as nucleophiles in the generation of thioether bonds with Iac F5 (iodoacetyl leaving group) and of disulfide bonds with PD F5 (pyridylthio leaving group). Second, if the surface is conjugated with 2,2′-dipyridyl disulfide, then the substrate becomes functionalized with leaving groups which can be displaced by Mac F5. The BTU and BATU surface-bound silanes can perform in an analogous electrophilic fashion to produce thioether links by displacement of the bromoalkyl and bromoacetyl leaving groups, respectively. The various reactions outlined above are summarized in Figure 2.

Reaction of Probes with the TTU Thiol-Functionalized Surface. Deprotected wafers were exposed to Iac F5, PD F5, and Iac F5 followed by Mac F5 under conditions where no effort was made to avoid contact with oxygen. The substrates were then studied in detail by X-ray photoelectron spectroscopy.[27] Although it is not possible to obtain an absolute value for the amount of surface-reacted probe using this technique, it is feasible to generate relative measurements of coverage. Accordingly, we compare the ratio of fluorine (from F 1s signals) to carbon (from C 1s signals) intensities found on an experimental level with those calculated on a theoretical basis for probe reaction with a layer of deprotected TTU and C8. This model computation takes the following form, where a 100% yield is arbitrarily assigned to the case where every active thiol group on the surface reacts with a probe molecule:

$$\frac{E_{XPS}}{C_{XPS}} = \frac{E_{probe}\, f_1 f_2}{(C_{probe}\, f_1 f_2) + (f_1 C_1 + (1 - f_1) C_2)} \quad (1)$$

In this equation, E_{XPS} and C_{XPS} represent the intensities to be expected from a unique element (for those probes, fluorine) and from carbon, respectively, E_{probe} and C_{probe} represent the numbers of fluorine and carbon atoms in the pentafluorinated probe, respectively, C_1 and C_2 are the numbers of carbon atoms in the active and diluent silanes, respectively, f_1 is the fraction of TTU silane in the TTU/C8 mixture, and f_2 refers to any fraction of any impurity silane that may be present. It should be noted that this

(27) Andrade, J. D. *Surface and Interfacial Aspects of Biomedical Polymers*; Plenum: New York, 1985; Vol. I.

Thioether Bond Formation
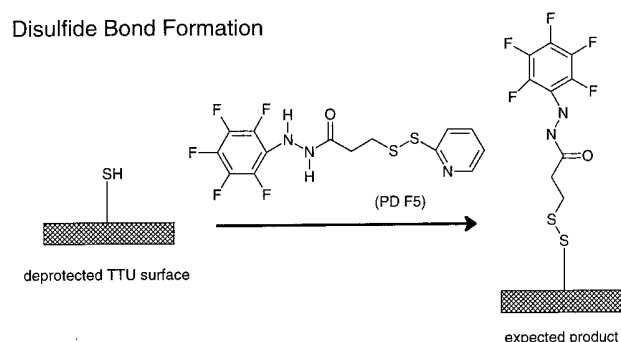

Disulfide Bond Formation

Figure 2. Surface conjugation reactions of probes with silanized surfaces.

Table 1. XPS F 1s/C 1s Intensity Ratios for 50% Deprotected TTU Surfaces Treated with Probes (O₂ Present)

probe and linkage	sample no.	exptl ratio[a]	exptl ratio[b]	calcd ratio (100% yield)	exptl yield, %
disulfide	1	0.040	0.036	0.180	20
PD F5	2	0.030	0.027	0.180	15
thioether	1	0.054	0.048	0.190	25
Iac F5	2	0.054	0.048	0.190	25
thioether−	1	0.049	0.043	0.190	23
disulfide	2	0.036	0.035	0.190	18
Iac F5−Mac F5					

[a] Fluorine contribution from both CF₃ and pentafluorophenyl groups.
[b] Fluorine contribution only from pentafluorophenyl group.

model assumes that competition for film growth between the active and diluent silanes is considered to be negligible. Furthermore, we have observed that deprotected TTU surfaces still contain about 10% of the original TFA functionalities after their removal. However, the pentafluorophenyl group is easily distinguished from the TFA group in the F 1s XPS spectrum.

The data for the experiments mentioned above are presented in Table 1. For the case of the formation of a disulfide linkage (PD F5), a yield of 17% is obtained, whereas the creation of a thioether bond (Iac F5) leads to a yield of 25%. If the latter surface is treated further with Mac F5 in a sequential manner, no additional loading occurs, as is evident from the overall value of 20% found for the yield in this experiment. An obvious explanation for this result is the formation of intractable disulfide bonds on

Table 2. XPS F 1s/C 1s Intensity Ratios for 50% Deprotected TTU Surfaces Treated with Probes (O₂ Free)

probe and linkage	sample no.	exptl ratio[a]	exptl ratio[b]	calcd ratio (100% yield)	exptl yield, %
disulfide	1	0.046	0.040	0.180	22
PD F5	2	0.040	0.035	0.180	19
thioether	1	0.191	0.184	0.190	97
Iac F5	2	0.155	0.150	0.190	79
reverse disulfide	1	0.009	0.007	0.190	3.7
DPDS[c]−Mac F5	2	0.023	0.018	0.190	9.5

[a] Fluorine contribution from both CF₃ and pentafluorophenyl groups. [b] Fluorine contribution only from pentafluorophenyl group. [c] 2,2′-dipyridyl disulfide.

the substrate surface through oxidative phenomena involving molecular oxygen. This constitutes a distinctively different mechanism from that where sulfonate formation occurs as a result of radiative catalysis.[28−30] The results for O₂-free conditions are shown in Table 2. As expected, Iac F5 now generates a dramatic improvement in yield, up from 28% to 88%. An XPS survey spectrum of the Iac F5−TTU deprotected system is shown in Figure 3A. A high-resolution XPS spectrum of the F 1s signal is shown in Figure 3B. Peak fitting can easily resolve the pentafluorophenyl peak (687.1 eV) from the TFA peak (690.1 eV). Only the pentafluorophenyl peak area was used for quantitation and assessment of the reaction yields for all probes, and this demonstrates the usefulness of these molecules for the case where residual TFA groups are still present on the surface. Figure 4A shows a peak-fitted N 1s spectrum. No reference for the hydrazide moiety of these molecules is available; therefore, the identification of the two peaks is based solely on the expectation that the pentafluorophenyl group is more electron-withdrawing than a carboxyl group. The ratio of the two peaks is not 1:1, as it should be; however, nitrogen is difficult to observe in this spectrum mainly because the low quantities present on the surface of the deprotected TTU−Iac F5 sample preclude an accurate analysis. Finally, Figure 4B depicts the carbon 1s high-resolution spectrum in which all of the functional groups are assigned. A summary of the high-resolution XPS analysis is given in Table 3.

The yield obtained with the PD F5 probe under oxygen-free conditions shows essentially no difference when compared to that obtained in the oxygen-abundant experiments (21% vs 19%). This can be explained through the assumption that PD F5 has the ability to induce surface disulfide formation preferentially through the thiol−disulfide interchange mechanism. The reaction of PD F5 with a surface thiol to form a PD F5−surface disulfide can be considered to be an intermolecular type of reaction. However, the reaction of an adjacent surface thiol with the PD F5−surface disulfide will displace the PD F5 group and create a surface−surface disulfide. This type of reaction is similar to an intramolecular process because the two adjacent surface thiols are confined in the self-assembled silane film environment, so that only the uppermost two or three carbons of each TTU molecule
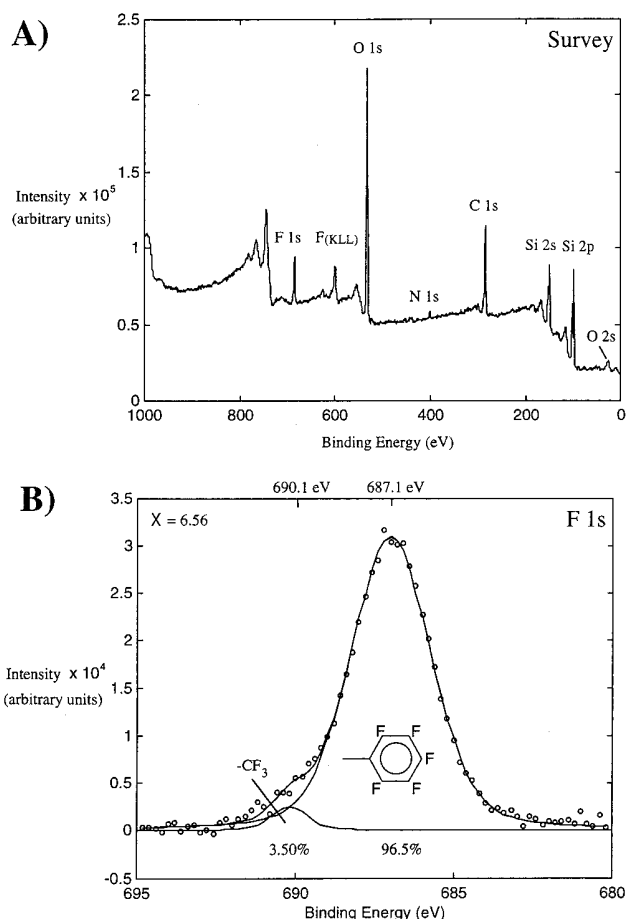

Figure 3. X-ray photoelectron spectra of 50% TTU/50% C8 after deprotection (removed of TFA group) and reaction with the probe Iac F5 (O₂-free conditions): A, survey spectrum; B, F 1s spectrum.

possess flexibility of movement. Therefore, surface−surface disulfide formation is somewhat analogous to creating a five- or six-membered ring, as does the reagent dithiothreitol (DTT). DTT has long been used as a reducing agent for intermolecular disulfides in solution because a six-membered ring can be formed when it is oxidized to form a disulfide bond. Accordingly, the equilibrium of the reversible reaction is strongly shifted toward intramolecular disulfide formation. Therefore, PD F5 acts as a catalyst for surface−surface disulfide formation, since it can only produce an intermolecular type of bond, and only isolated thiols on the surface will form stable PD F5 adducts. Oxygen- and disulfide-interchange-induced formations of surface disulfide linkages are depicted in Figure 5.

A "reverse" approach to disulfide conjugation was also attempted using the probe 2,2′-dipyridyl disulfide. Treatment of the substrate surface with this compound should create a mixed alkyl 2-pyridyl disulfide. In this circumstance, the leaving group for disulfide formation is located on the surface, and the thiol-containing probe Mac F5 was used in an attempt to create a disulfide link to the surface through the thiol−disulfide interchange reaction. Even though this reaction was performed under oxygen-free conditions, the observed yield was 7% (Table 2), which is much lower than that of the reverse situation, where PD F5 contained the leaving group and the thiol was located on the surface. Steric hindrance seems to be the key factor responsible

(28) Thompson, M.; Whelan, J.; Zeman, D. J.; Bosnich, B.; Solomon, E. I.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 2482.
(29) Thompson, M.; Lennox, R. B.; Zenon, D. J. *Anal. Chem.* **1979**, *51*, 2260.
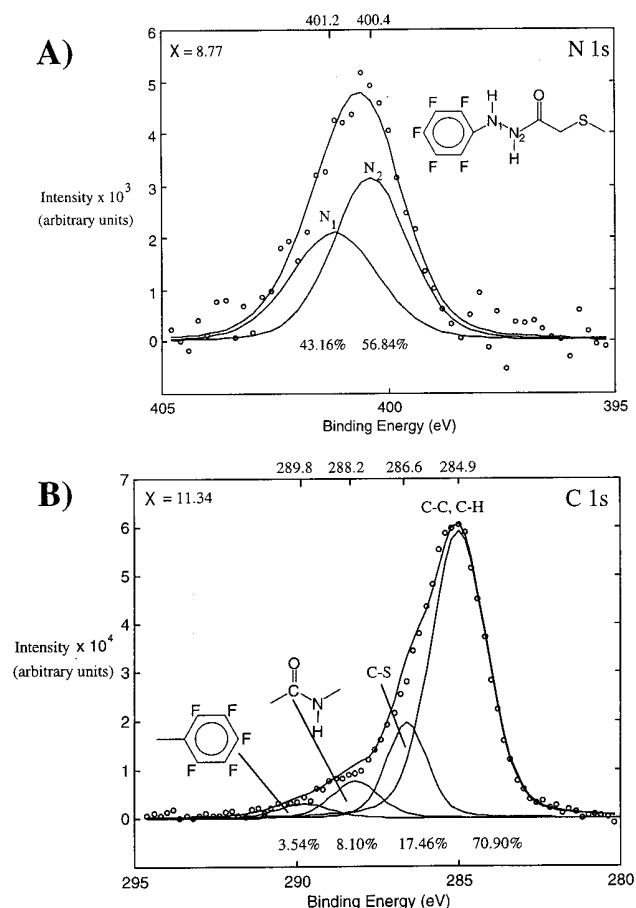(30) Bathia et al. *Anal. Biochem.* **1993**, *208*, 197.

Figure 4. X-ray photoelectron spectra of 50% TTU/50% C8 after deprotection and reaction with the probe Iac F5 (O$_2$-free conditions): A, N 1s spectrum; B, C 1s spectrum.

Table 3. High-Resolution XPS Analysis of 50% Deprotected TTU Surfaces Treated with Iac F5 (O$_2$ Free)

signal	binding energy, eV	atom wt %	ident	χ
F 1s	687.1	96.5	C$_6$F$_5$	6.56
	690.3	3.5	CF$_3$	
N 1s[a]	400.4	56.8	N2	8.77
	401.2	43.2	N1	
C 1s	284.9	70.9	C−C	11.34
	286.6	17.5	C−S	
	288.2	8.1	−COH−	
	289.8	3.5	C$_6$F$_5$	
Si 2p	99.2	67.8	Si^0	11.10
	103.0	32.2	SiO$_2$	

[a] As assigned in Figure 4A.

for this result, since Mac F5 must penetrate past the pyridyl group in order to attack the surface thiol group. In the reverse situation, PD F5 could adopt a wide range of conformations in which the pyridyl leaving group is orientated away from the surface and steric effects are minimized. This is a novel finding because pyridyl leaving groups have been used previously in a wide variety of disulfide-based immobilization schemes.[13,28] However, in most of these systems, the pyridyl leaving group is attached to the end of
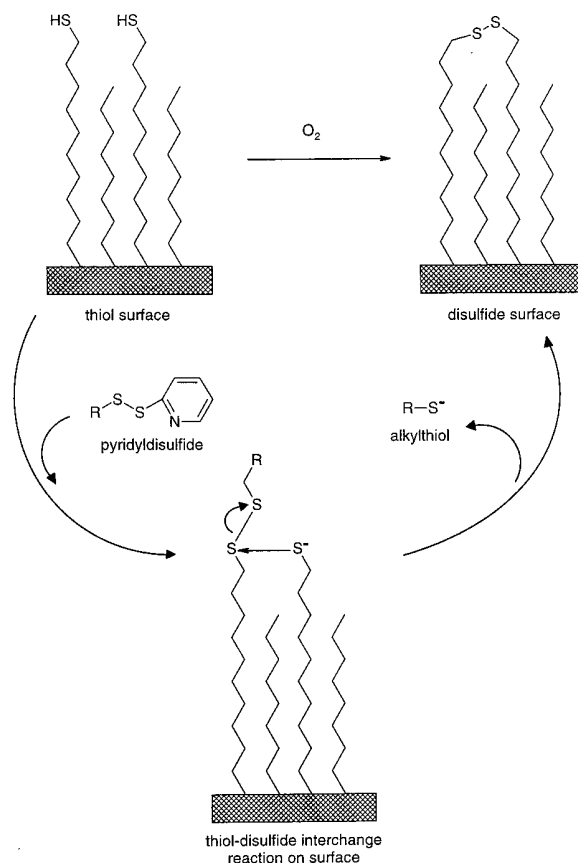

Figure 5. Mechanisms for disulfide formation on silanized surfaces: A, oxygen mediation; B, thiol−disulfide interchange reaction.

Table 4. XPS F 1s/C 1s Intensity Ratios for 50% BTU and BATU Surfaces Treated with Mac F5

surface	sample no.	exptl ratio	calcd ratio (100% yield)	exptl yield, %
BTU	1	0.008	0.190	4.2
BTU	2	0.008	0.190	4.2
BATU	1	0.040	0.140	29
BATU	2	0.050	0.140	36

a much longer chain than used in our experiments and is in a significantly more flexible situation. Additionally, many of the tethers used to anchor the disulfide to the surface form highly disordered multilayers, which again would provide the pyridyl group freedom for movement. Therefore, the experiment involving surface thiol reaction with 2,2′-dipyridyl disulfide followed by Mac F5 constitutes a system with high steric hindrance caused by surface crowding, and accordingly, represents a special case.

Reactions of Surface-Immobilized BTU and BATU. Surfaces silanized with BTU and BATU can both behave in an electrophilic manner in their reactions with the probe Mac F5 since they contain the reactive leaving moieties bromide anion and bromoacetate, respectively. X-ray photoelectron spectroscopic analysis of the products of these reactions performed analogously to those described for TTU and BTU above is given in Table 4. Clearly, BTU generates a very low yield (4%), while in contrast, the more reactive BATU reacts with Mac F5 resulting in a yield of 33%. A key factor here is that BTU has three more carbon atoms
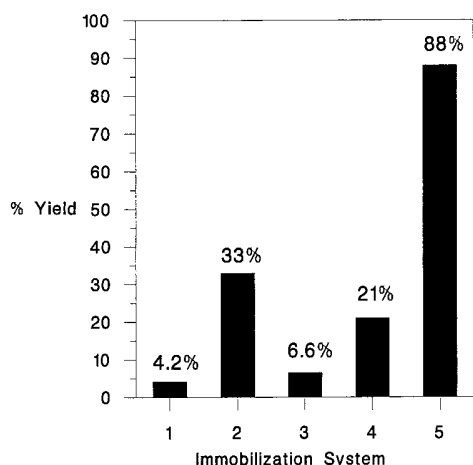
Figure 6. Immobilization of fluorinated probes on surfaces with attached deprotected TTU, BTU, and BATU. A yield of 100% represents a maximum quantity of probe loaded for the particular silane system. Key to reactions: 1, BTU surface with Mac F5; 2, BATU surface with Mac F5; 3, TTU surface with 2,2′-dipyridyl disulfide followed by Mac F5; 4, TTU surface with PD F5; TTU surface with Iac F5.

than C8 (the diluent), whereas BATU possesses six more atoms than the same molecule. Accordingly, it is possible that this structural difference is responsible for the relief of steric hindrance in the BATU system compared to the BTU chemistry. A comparison of the BATU system with the other systems described in the present paper (Figure 6) indicates that BATU immobilized on substrate surfaces could prove to be useful in the attachment of nucleophilic bimolecules at interfaces. However, the yield for the BATU system is significantly lower than that for the TTU−Iac F5 case where the electrophilic entity is the probe molecule. The small difference in reactivity between iodoacetates and bromoacetates is insufficient to account for the 55% discrepancy between these two systems. Bromoacetates are light sensitive, more so than alkyl bromides, and it is possible that exposure to light may cause enhanced loss of bromine in the BATU system. However, this factor was considered carefully in the experimental arrangements, and more importantly, Iac F5 is significantly light sensitive but still produces the highest yield in reactions with nucleophilic thiol groups on the substrate surface. Accordingly, it is clear that important considerations in all these surface chemistries are the location of the leaving group and steric factors.

It appears to be advantageous to attach leaving groups to probe molecules in bulk solution, where they are obviously in vast excess over the number of thiol functionalities present in the substrate surface. If light sensitivity constitutes a critical element in these reactions, loss of activity caused by surface exposure to radiation is likely to be much more serious than if the exposure occurs in bulk solution.

CONCLUSIONS

Thiol functionalities can participate in both disulfide and thioester bonding whether placed on the substrate surface or at the end of a biomolecule such as an oligonucleotide. The disulfide bond can be formed using the highly specific thiol−disulfide exchange process which occurs via an S_N2 mechanism in water at neutral pH. Thioester bonds can be created with facility through the use of reagents which contain reactive leaving functionalities. The bifunctional silanes discussed in the present work can act in both electrophilic and neutrophilic capacities; for example, surface-bound BATU can react with nucleophilic species in the release of the bromoacetate ion or can be modified to produce a thiol-functionalized surface. This portfolio of combinations allows the possibilities of permanent or reversible attachment to nucleic acid end-chain groups in a relatively mild environment. Furthermore, the bound species are highly stable to chemical and thermal attack, an important consideration at annealing temperatures.

SUPPORTING INFORMATION AVAILABLE

Text presenting synthetic protocols for *N*-(pentafluorophenyl)-3-(2-pyridyldithio)proprionohydrazide, *N*-(pentafluorophenyl)-iodoacetohydrazide, *N*-(pentafluorophenyl)-mercaptoacetohydrazide, *ω*-undecenyl bromoacetate, and 1-((bromoacetyl)oxy)-11-(trichlorosilyl)undecane. This material is available free of charge via the Internet at http://pubs.acs.org.