Silica Surface Modification and Its Application in Permanent Link with Nucleic Acids

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ABSTRACT: In this paper, the Pt-catalyzed hydrosilylation of hydroxyl ethers is described. Various bifunctional alkoxysilanes were obtained and applied in O-silylation of free hydroxyl groups on the silica surface. These modified solid materials have been used as excellent supports for linking synthetic nucleic acids. Nucleic acids permanently attached to the solid surface were tested in hybridization with complementary fluorescence-labeled sequences. Detection of nucleic acids anchored to the solid support was performed by fluorescence microscopy after hybridization.

INTRODUCTION

Hydroelementation of unsaturated organic compounds is one of the most important and atom-efficient method for the incorporation of heteroatoms into various unsaturated systems.1-7 Indeed, the hydrosilylation reaction8-12 provides access to synthetically useful organofunctional silanes,13-20 which are widely used as synthetic intermediates in several processes. Alkoxysilanes play a significant role in the synthesis of siloxanes21 and for the preparation of novel, functionalized silicas which are attractive because of their practical applications.22 The structure of alkoxysilanes, especially their dual functionality, allows well-defined and unique materials to be obtained after the spontaneous condensation reaction that takes place on the material’s surface (Figure 1).

Nowadays, coating silica particles with alkoxysilanes by the formation of strong covalent Si–O–Si bonds to such surfaces23,24 leads to a large number of new interesting applications.25,26 One of these is solid supports [e.g., controlled pore glass (CPG) silicas,27 as well as polymeric and hybrid ones28,29], which allow nucleic acids to be synthesized on solid phase but with solution interaction. Using CPG gives a larger surface for growing the oligonucleotide chain.30 The availability of the surface is vital; however, the properties of linker and surface chemical functionality allow for finding proper applications. To link biopolymers, amines31,32 epoxide,33 azide,34 hydroxyl,35 or thiol36 functional groups are mainly used. In practice, a removable linker is used in nucleic acid synthesis at the solid phase, but in diagnostic applications, nucleic acids need to be permanently linked to the solid surface.36

The chemical linker for the permanent attachment of the synthetic nucleic acids to the solid support must be very resistant in base or acid hydrolytic conditions, which are used to completely remove protecting groups. The nucleic acid was attached to the support by phosphate linkage using phosphoramidite chemistry.38 Polypropylene39 and polysyrene40 are currently the most commonly used as supports for permanent anchored oligonucleotides. However, a more and more popular functional surface used in phosphoramidite chemistry is functional glass surfaces because the swelling of the support (observed for polymers) is eliminated. However, to permanently anchor the oligonucleotide on the glass surface, new modifications need to be applied due to the fact that the linkers commonly used on functional surface are removable. For this purpose, chemical modification of the glass support with new organofunctional silanes was applied, which may produce resistant linkers that permanently anchor oligonucleotides. Alkoxysilanes (i.e., 3-glycidoxypropyl trimethoxysilane) were used as hydroxy functional linkers, but these are largely unstable in base hydrolysis conditions (hydroxyl ammonium, temp. 55 °C). However, such conditions are necessary for the removal of protecting groups from the oligonucleotide after chemical synthesis. This resulted in the removal of the oligonucleotide from the support. Other linkers such as 3-[bis(2-hydroxyethyl)amine]propyltriethoxysilane and N-(3-triethoxysilylpropyl)-4-hydroxy-butryamide40 were applied to
short oligonucleotides were used. 

Veri links to be used later in automated oligonucleotide syntheses. We tested mono-, di-, and tri- supports that would allow for the permanent linking of synthetic nucleic acids. We tested mono-, di-, and tri-ethoxysilanes, which enabled selection of the most permanent synthetic challenge. Herein, we report the highly selective synthesis of bifunctional alkoxysilanes via the hydroxyalkylation reaction. They were subsequently applied to silanization of the CPG silica surface, which yielded new supports that would allow for the permanent linking of synthetic nucleic acids. We tested mono-, di-, and tri-alkoxysilanes, which enabled selection of the most permanent links to be used later in automated oligonucleotide syntheses. The stability and functionality of these links were further verified in hybridization experiments where fluorescently tagged short oligonucleotides were used.

### RESULTS AND DISCUSSION

The first goal was to obtain alkoxysilanes with hydroxyl functional groups. It is commonly known that this may increase the availability, flexibility, and reactivity of such groups. As a starting point, the reactions between unsaturated ethers containing OH groups with various ethoxysilanes were considered. Unfortunately, the presence of the free hydroxyl moiety significantly hindered the hydroxyalkylation with alkoxysilanes. Because of this, it was decided to temporarily protect all OH groups with a trimethylsilyl (TMS) group. In practice, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) has been used as a TMS donor because gaseous ammonia is the only byproduct. To increase the silylating potential of HMDS, numerous catalysts and activators have been tested.43,44 One of these are metal triflates,43,44 which attract special attention because of their high catalytic activity in various organic transformations.45-50 It is worth noting that inexpensive Bi(OTf)₃ is the most effective in this process. It was selected as the catalyst in the silylation reaction (Scheme 1). It is worth reiterating that this procedure has been used by us in our previous research.15 Structures of synthesized products are summarized in Figure 2. All of them are known in the literature.

O-silyl protected unsaturated ethers (1–5) were subjected to catalytic combination of various mono-, di- and trialkoxysilanes and [Pt₃{(Me₂SiCH=CH₂)₂O}] in toluene at 60 °C under argon atmosphere (Scheme 2). All of these were successfully hydrosilylated dominantly in the anti-Markovnikov fashion. Table 1 summarizes the results for these reactions. Thereafter, mono-, di- and tri-ethoxysilanes with one or two −OSiMe₃ groups (products: 8, 10, 13, 14, 17, and 19) were selected for further work on anchoring nucleic acids to CPGs. The above-mentioned alkoxysilanes were used in the silanization of CPG silicas (Figure 3).

CPG supports result from etching the surface; hence, their pore sizes are estimated. Silanization may change the structure of the surface; therefore, silane with low concentration values should be used. An optimal surface was selected from surfaces with different pore sizes (CPG 1000, CPG 1500, CPG 2000, and CPG 3000). Selected silane (Table 1, entry 12; concentration = 1%) was further applied in silanization of the above-mentioned CPG silicas. After that we tested so modified CPGs on automated DNA/RNA synthesizer attaching d(T)₄ in four synthetic cycles collecting and quantifying the dimethoxystyryl (DMT) cation after last cycle. The measured absorbance of DMT allowed us to choose CPG 1500 as the most optimal one, which was characterized by the high level of covalently linked nucleic acid. Next, all of the selected bifunctional silanes were used in silanization of CPG 1500.

The appropriate silane at various concentrations (1 and 2%) in toluene was stirred with CPG for 24 h. After that, the silica was dried for 5 h at 110 °C. It is known that some of the surfaces’ hydroxyl groups could be not silylated by alkoxysilanes. Thereafter, to block all hydroxyl groups, the modified support was stirred for 24 h in 15% solution of HMDS in toluene. This procedure of protection was carried out to be sure that the surfaces’ hydroxyl group would not interrupt the nucleic acid immobilization.

The surfaces prepared in accordance with the above-mentioned procedure were applied in further immobilization of nucleic acids. A loading level of covalently linked biomolecules was evaluated by the measurement of DMT absorbance before and after the use of basic conditions (Table 2, all entries). In the case of silicas modified by monoalkoxysilanes (Table 2, entries 1–8), the highest loading of biomolecules was observed for silanes with higher concentration (2%; Table 2, entries 3–4 and 7–8). However, with
regard to stability in basic conditions, the most optimal were surfaces modified by less concentrated solutions (1%; Table 2, entries 1−2 and 5−6). In the case of silanes with two alkoxy groups (Table 2, entries 9−16), in all cases the level of linked oligonucleotides was good, but increased linker stability was observed when the support was additionally treated by HMDS.
The surfaces modified by trialkoxysilanes were also characterized by a high level of loading. However, the stability after ammonium treatment was extremely low (Table 2, entries 17−20 and 23). As it took place in previous tests (Table 2, entries 5−6 and 13−14), here too the surfaces modified by alkoxy silanes with two hydroxyl groups were the most stable (Table 2, entries 21 and 22). The results presented in Table 2 show that using of HMDS during CPG support preparation increases the yield of oligomer bonding because the accessibility of free hydroxyl groups for the reaction with nucleoside phosphoramidite is higher. We also observed that HMDS prevents the removal of nucleic acid during the deprotection step (treatment by ammonium).

Seeing that HMDS-modified supports reveal increased the stability of oligomer bonding in a basic environment, they were used in longer nucleic acid synthesis. To this end 70 mer seq1 on support D (1% + HMDS) and 26 mer seq2 on the commercial support were synthesized. Seq2 is fluoresceine-labeled and its sequence is selected to be complementary with part of seq1. Next, a hybridization of the support with seq1 with the probe seq2 was performed in a hybridization buffer within 1.5 h in 80 °C. As a reference sample to eliminate the nonspecific effect of the support with the probe, we performed hybridization with supports on which synthesis of seq1 was not performed. After washing, the supports were analyzed using fluorescent microscopy. The image presented in Figure 4 clearly shows that in the case of supports silanized by D (1% + HMDS) the seq1 oligomer is bonded to the support and interacts with a fluorescent probe. The effect is not visible on supports without seq1.

Figure 4. Images from fluorescent microscope presenting the CPG support. The left image of CPG is after synthesis of 70 mer DNA and hybridization with a fluorescent probe. The right image represents CPG without seq1 only after hybridization with a fluorescent probe.

CONCLUSIONS

To sum up, we report a highly selective synthesis of bifunctional alkoxy silanes via the hydrosilylation reaction, which were further applied in silanization of the CPG silicas.

Table 2. Comparison of Stability of Bonding Oligonucleotide with the Support and Influence of Type of Silanes, Concentration, and HMDS on Stability of the Bonding after Ammonium Treatment

| entry | silane b (concentration) | stability before ammonium (μmol/g) | stability after ammonium (μmol/g) | % a  |
|-------|-------------------------|-----------------------------------|-------------------------------|-----|
| 1     | A (1%)                  | 12.69                             | 9.4                           | 74.1|
| 2     | A (1% + HMDS)           | 14.17                             | 12.39                         | 87.4|
| 3     | A (2%)                  | 15.11                             | 2.7                           | 17.9|
| 4     | A (2% + HMDS)           | 16.82                             | 6.3                           | 37.5|
| 5     | B (1%)                  | 21.95                             | 18.4                          | 83.8|
| 6     | B (1% + HMDS)           | 19.91                             | 17.54                         | 88.1|
| 7     | B (2%)                  | 21.62                             | 8.19                          | 37.9|
| 8     | B (2% + HMDS)           | 23.79                             | 14.2                          | 59.7|
| 9     | C (1%)                  | 22.11                             | 8.66                          | 39.2|
| 10    | C (1% + HMDS)           | 20.16                             | 10.83                         | 53.7|
| 11    | C (2%)                  | 25.01                             | 7.56                          | 30.2|
| 12    | C (2% + HMDS)           | 26.10                             | 11.54                         | 44.1|
| 13    | D (1%)                  | 21.94                             | 18.02                         | 82.1|
| 14    | D (1% + HMDS)           | 24.86                             | 20.01                         | 81.3|
| 15    | D (2%)                  | 25.38                             | 10.35                         | 40.8|
| 16    | D (2% + HMDS)           | 26.30                             | 19.76                         | 75.1|
| 17    | E (1%)                  | 21.44                             | 7.52                          | 35.1|
| 18    | E (1% + HMDS)           | 23.83                             | 6.3                           | 26.4|
| 19    | E (2%)                  | 18.03                             | 3.01                          | 16.7|
| 20    | E (2% + HMDS)           | 25.90                             | 6.19                          | 23.9|
| 21    | F (1%)                  | 21.96                             | 17.60                         | 80.1|
| 22    | F (1% + HMDS)           | 24.89                             | 19.72                         | 79.2|
| 23    | F (2%)                  | 32.49                             | 7.8                           | 24.0|
| 24    | F (2% + HMDS)           | 38.52                             | 19.18                         | 49.8|

aPercentage of permanent linking of synthetic nucleic acid. bStructures of silanes used for silanization of CPG silica:

(Table 2, entries 10, 12, 14, and 16).
The choice of efficient solid support, as well as a stable linker on its surface still remains a significant challenge in the synthesis of nucleic acids at the solid phase. In this paper, we have demonstrated that the presented CPG supports with linkers bearing hydroxyl groups are effective in the chemical synthesis of nucleic acids. What is more, the deprotection of protecting groups (by means of ammonium) does not result in their complete removal from the solid surface. The thus-obtained oligonucleotides retain their natural capability of forming such secondary structures as duplexes. The observed partial removal of oligonucleotide by ammonium is probably connected with removing a layer of silane with the bonded oligonucleotide. In all cases, the best results were achieved when the silane concentration (regardless of the number of alkoxy groups) was at 1%. Monoethoxysilanes, as well as diethoxysilanes, have been shown to give the most stable monolayers, especially for linkers bearing two hydroxyl groups. The results obtained for trialkoxysilanes were more diversified; the presence of three alkoxy groups may probably cause additional cross-linking between them, which prevents the formation of stable monolayers.

## MATERIAL AND METHODS

### General Information.**

The reagents and bismuth(III) triflate used for experiments were purchased from Sigma-Aldrich Co. and used without further purification. HMDS was purchased from ABCR GmbH & Co. KG and used without further purification. CPG silica was purchased from BioTech Technology. All data connected to the synthesis products are presented in the Supporting Information file. **H NMR (400 MHz) and 13C NMR (101 MHz) were recorded on a Bruker AVANCE III HD NanoBay (600 MHz) spectrometer using CD3OD as the solvent. GC analyses were performed on a Varian 3400 with a Megabore column (30 m) and TCD. The mass spectra of products were determined by GC–MS analysis on a Varian Saturn 2100T, equipped with a 45-μm capillary column (30 m) and Finnigan Mat 800 ion trap detector. Absorption was measured by Jasco V-650 spectrometer at 498 nm. The spectra of products were determined by GC–MS analysis on a Varian Saturn 2100T, equipped with a 45-μm capillary column (30 m) and Finnigan Mat 800 ion trap detector. Absorption was measured by Jasco V-650 spectrometer at 498 nm.

### Representative Procedure for the Silylation of Various Unsaturated Alcohols (1–5).

To one 100 mL one-necked round-bottom flask, unsaturated alcohol (0.23 mol), HMDS (0.115 mol), and Bi(OTf)3 (0.0001 mol) were added. The reaction mixture was stirred at room temperature for a defined time. The reaction progress was monitored by GC analyses. After the reaction was complete, the crude product was purified by trap-to-trap distillation to give the corresponding silylated alcohols (1–5).

### Representative Procedure for the Hydroisilylation (6–19).

To one 100 mL one-necked round-bottom flask, unsaturated alcohol (0.029 mol), alkoxysilane (0.034 mol), and Karstedt catalyst (0.028 mmol) were added. The reaction mixture was stirred at 60 °C for 6 h. The reaction progress was monitored by GC and GC–MS analyses. After the reaction was complete, all volatiles were removed under reduced pressure. The crude products were purified by trap-to-trap distillation to give the corresponding silylated alcohols (6–19).

### Functionalization of CPG.

The appropriate amount of alkoxysilanes, 0.5 g of CPG-1500, and 25 mL of toluene were placed in a one-necked round-bottom flask. The mixture was stirred at room temperature for 24 h. Next, the functionalized CPG was filtrated and washed with toluene (4 × 25 mL) and dried in 110 °C for 5 h. Thereafter, a 15% solution of HMDS in toluene was added to the modified support and it was stirred at room temperature for 24 h. After the filtration procedure, the material was washed with toluene (2 × 25 mL) and dried in 110 °C for 5 h.

### Immobilization of Nucleic Acids. Part 1—Deprotection with the Acid.

The support was weighed (approx. 100 mg) and transferred to a vial by adding 1 mL of 0.1% HCl and 0.5 mL of acrylonitrile and placed in the incubator for 1 h at 50 °C. Then, each sample was washed successively with 0.1% HCl, H2O Milli-Q, methanol, and diethyl ether and then transferred back into vials and placed in the incubator for 2 h at 80 °C.

### Part 2—Synthesis.

The substrates were weighed (approx. 15 mg) and placed in small columns, and the synthesis was performed on them 4-mer deoxythymidine d(TTTT) without removing the last DMT groups. Phosphoramidite used in the reaction are d-T-CE dissolved in dry acetonitrile (POCH). The synthesis was performed on a scale of 0.2 μmol for a phosphoramidite condensation time of 5 min. The reagents used in the reaction were 3% dichloroacetic acid (DCA), dehydrated acetonitrile (POCH), Hyacinth benzylmercaptotriazole (BMT) solution—0.25 M BMT (empBIOTECH), oxidizing solution OXI [iodine/water/pyridine/tetrahydrofuran (THF)], and capping reagent (acetic anhydride/pyridine/THF); the second capping reagent (1-methylimidazole/THF) was purchased from Roth. After the synthesis, the synthetic nucleic acid was dried and divided into two portions, the first portion was weighed and 3% DCA was added, after that the absorption was measured and the load level was calculated. Ammonia (32%) was added to the second part of the substrate and left for 30 min at rt. This was then added to the solution from the substrate and the substrate itself was also carefully dried and measured for absorption.

### Hybridization.

Supports with and without oligonucleotides (3 mg) were suspended in 200 μL of hybridization buffer [5× SSC + 0.1% sodium dodecyl sulfate (SDS) + 0.1 mg/mL bovine serum albumin]. The seq2 (11 OD) dissolved in 200 μL of water was heated to 80 °C (5 min) and 8 μL was added to the support and kept at room temperature for 1.5 h. After this time, the support was washed in a 3× 1 mL buffer (2× SSC + 0.1% SDS). The support was dried in a closed dish under reduced pressure overnight.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00547.

NMR data and spectra of compounds synthesized in this paper (PDF)

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Heteroatom (N, P, O, S) Bonds.

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Notes

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