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Immobilization of biomolecules via ruthenium-catalyzed functionalization of the surface of silica with a vinylsilane *

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ABSTRACT

A new ruthenium complex catalyzed procedure for the efficient *O*-silylation of an SiO–H group on the silica surface of glass beads with controlled pores (CPG) using 1-trimethylsiloxy-3-dimethylvinylsilylpropane leading to Si–O–Si bond formation with the evolution of ethylene is described. The formed linker contains alkyl hydroxyl groups which can be reacted with a nucleoside phosphoramidite unit. The CPG support containing alkyl hydroxyl groups is successfully applied in automatic chemical synthesis of DNA fragments.

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Surfaces of silica or glass modified with organic functional groups have received considerable attention in materials science. They are widely applied in biopolymer synthesis as solid supports,¹ microarray surfaces,² and as chemical carriers.³ Most silica surface modification methods are based on the reaction between Si–OH groups of the surface with alkoxy-, chloro-, or acyloxysilanes.⁴ However, these unstable and reactive silylating reagents are sensitive to moisture, cannot be handled under hydrolytic conditions and cannot be purified by silica gel chromatography. To overcome these difficulties, allylsilanes have been applied recently as moisture-stable silylating reagents for silica surface functionalization.^{5,6} Jun and Park have developed a new grafting methodology based on the iridium-catalyzed reaction of vinylsilanes with silica or glass surfaces.⁷

The silylative coupling of olefins with vinyl-substituted organosilicon compounds, which we have previously developed as a new, effective catalytic method for the activation of the =C–H bond of olefins and =C–Si bond of organosilicon compounds (generally occurring in the presence of complexes containing M–H and M– Si bonds),^{8,9} has recently been found to be general and also exhibited by vinyl derivatives of other *p*-block elements (e.g. boron¹⁰ and germanium.¹¹) This mode of catalytic reactivity has also been successfully extended to catalytic activation of other sp² carbons, i.e. $=C_{aryl}-H$ bonds¹² and sp-hybridized carbon-hydrogen bonds,¹³ as well as by the O-H bonds of silanols,¹⁴ alcohols¹⁵ and boronic acids¹⁶ (Scheme 1).

The mechanism of this general reaction, in which vinyl metalloid compounds ($CH_2=CHER_n$) act as metallating agents and hydrogen acceptors, has been proved to involve the insertion of the vinyl metalloid compound into the TM–H bond (where TM = Ru, Rh, Ir or Co), and β -metalloid transfer to the transition metal with elimination of ethylene and generation of a TM–E (E = Si, Ge, B) bond. In the next step, migratory insertion of a coupling substrate (alkene, alkyne) or oxidative addition of a compound containing an –OH group into the TM–E bond followed by β -hydride transfer to the metal (or reductive elimination) eliminates the metallated product.⁸

In view of our recent reports on the successful use of vinylsilanes, vinylboronates, and vinylgermanes as new hydrogen acceptors for the *O*-metallation of silanols,^{14a-c} the aim of this work was to check the possibility of extending the role of vinylsilanes as silylating agents in the *O*-silylation of hydroxyl functional groups on the surface of silica. This has great potential in the hydrophobization strategy of inorganic (and organometallic) materials, and is more and more widely applied in the synthesis of biomolecules such as nucleic acids.

Herein, we report a new and complementary catalytic method of functionalizing a silica surface, which involves activation of

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G. Hreczycho et al. / Tetrahedron Letters xxx (2013) xxx-xxx

$$R-X-H + H_2C \stackrel{H}{\sim} ER'_n \xrightarrow{TM cat.} R-X-ER'_n + CH_2=CH_2$$

 $X = HC = CH, C \equiv C, O, or Ar$

ER'n = SiR3, GeR3, BR2, Ar, R3C

R = R' = alkyl, aryl, siloxyl

Scheme 1. Catalytic metallation by deethenative coupling with vinyl metalloids and concomitant elimination of ethene.



Scheme 2. Silica surface modification using a vinylsilane.



Scheme 3. Synthesis of 1-trimethylsiloxy-3-dimethylvinylsilylpropane.

the O–H bond on the surface by a moisture-unstable vinylsilane occurring in the presence of a ruthenium hydride catalyst via elimination of ethylene (Scheme 2). The prepared functionalized support with alkyl hydroxyl groups in the presence of ruthenium complexes 1-3 was used in automated chemical synthesis of nucleic acids. The obtained nucleic acid was subsequently able to hybridize with a complementary fluorescent probe. The nucleic acid chain grows freely on a functionalized support if: (i) it has enough space for growing the chain, and (ii) the linker connecting it with the support is stable under the synthetic conditions.

We chose 1-trimethylsiloxy-3-dimethylvinylsilylpropane **4** as an organo-functionalized vinylsilane, because the formed link is expected to be stable under the conditions of nucleic acid synthesis, and provides sufficient distance between the hydroxyl groups and the surface that enhances space for the growing chain. Compound **4** was prepared from commercially available allyl alcohol by using a sequential procedure: alcohol silylation – Pt-catalyzed hydrosilylation – Grignard substitution, in high overall yield (73%), as outlined in Scheme 3.¹⁷

Ruthenium(II) and ruthenium(0) complexes, i.e. [(RuHCl(-CO)(PCy₃)₂] **1**, [Ru₃(CO)₁₂] **2** and [Ru(COD)(COT)] **3**, which are known to be active in *O*-silylations of silanols with vinylsilanes^{14a} and vinylgermanes^{14b} were tested for the *O*-silylation of the hydro-xyl groups of the silica surface with 1-trimethylsiloxy-3-dimethyl-vinylsilylpropane **4**. All the reactions were conducted following the original procedure for the synthesis of unsymmetrical siloxanes^{14a} in the presence of 2 mol % of the catalyst with 2–4 equiv of the ole-fin in a Schlenk bomb flask fitted with a plug valve in toluene (110 °C). The effectiveness of the catalytic method was shown by functionalizing glass beads with controlled pores (CPG) and applying them in chemical syntheses of nucleic acids. The silyl ether



Scheme 4. Method for testing the solid CPG with a phosphoramidite unit in the chain extension reaction.

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G. Hreczycho et al. / Tetrahedron Letters xxx (2013) xxx-xxx

Entry	Catalyst	Molar ratio (CH ₂ =CHSiR ₃):(HO)	Time (h)	Loading (μ mol g $^{-1}$)
1	$(RuHCl(CO)(PCy_3)_2]$ 1	4:1	4	30.21
2	$(RuHCl(CO)(PCy_3)_2]$ 1	4:1	12	42.11
3	$(RuHCl(CO)(PCy_3)_2]$ 1	4:1	17	48.8
4	$[Ru_3(CO)_{12}]$ 2	2:1	24	19.79
5	Ru(COD)(COT)] 3	2:1	24	24.16

Loading efficiency of glass beads via O-silylation of an Si-O-H group on the CPG silica surface with vinylsilane 4 under different reaction conditions

Reaction conditions: toluene (0.1 M), 110 °C, catalyst loading – 2 mol $\%^{18}$

Table 1

groups formed on the glass bead surface in the *O*-silylation process have a hydroxyl group blocked with trimethylsilyl. It is possible to remove this group by treatment with water in acidic conditions, which leads to a primary hydroxyl group. This hydroxyl group can then react with a phosphoramidite unit, extending the nucleic acid chain (Scheme 4). Removal of the 4,4'-dimethoxytrityl group (DMT) after each step of the chain extension using a solution of weak acid produces a colored cation characterized by a maximum absorption at a wavelength of 504 nm. Thus, we can determine the molar quantity of hydroxyl groups per unit area (Scheme 4). On the other hand, we can define the quantity of available hydroxyl groups based on their reactivity.

Ruthenium(0) and especially ruthenium(II) hydride complexes are known to be active in O-silylation of silanols with vinylsilanes, but are also good catalysts in silylative homocoupling of vinylsilanes. Therefore, in the reaction of 1-trimethylsiloxy-3-dimethylvinylsilylpropane with the O–H bonds on the silica surface in the presence of **1–3** catalysts a two or fourfold excess of vinylsilane was necessary to achieve the maximum modification of the silica surface.

The quantitative measurement of DMT cation concentration during the process of extending the nucleic acid chain on the support (after *O*-silylation with vinylsilane **4**) enables optimization of the proportions of the applied catalyst and the time of *O*-silylation. This should produce supports with the highest concentration of effective hydroxyl groups (Table 1). The parameter characterizing such a support is the estimated level of loading of the surface with hydroxyl groups.

Although the excess of vinylsilane in the presence of ruthenium complexes facilitates competitive formation of the vinylsilane homocoupling product, its use also enables a maximum loading of the silica surface by silyl ether groups. Moreover, the accompanied bis(silyl)ethenes can be easily separated by simple filtration of the modified silica.

The loading efficiencies were between 19.79 and 48.8 μ mol g⁻¹ depending on the time and the catalyst¹⁹. All the tested complexes exhibited catalytic activity. The reaction was catalyzed effectively by **1** in toluene, and after 17 h with 2 mol % of catalyst the loading was 48.80 μ mol g⁻¹.

Thus the functionalized support (Table 1, entry 3) does not limit the growth of oligonucleotides up to 50-mer and may be used in applications which require oligonucleotide stability, because the oligonucleotide is permanently linked to the support surface after the synthesis is completed. To demonstrate the utility of oligonucleotides on the support, a duplex with a complementary label DNA strand was formed. Figure 1 depicts the result of hybridization between the oligonucleotide linked to the support and the complementary fluorescent probe.²⁰

The CPG beads on which the synthesis was not performed were used as a reference. They are unable to interact with the fluorescent probe. Hence, only the CPG beads containing the oligonucleotide emit fluorescence resulting from the pairing of two complementary DNA strands, one of which is a fluorescent probe. The picture below presents both supports: fluorescence-emitting and a reference.



Figure 1. Two types of CPG beads are depicted. Chemical synthesis of DNA (ODN-1) was performed only on one of them. However, both were included in the hybridization process with a complementary fluorescent probe (ODN-2). Green fluorescence was visualized using a fluorescent microscope after removing the reaction residues. Microscopic magnification: $200 \times$.

In conclusion, we have developed a new catalytic route for efficient *O*-silylation of hydroxyl groups on the surface of silica with moisture-unstable 1-trimethylsiloxy-3-dimethylvinylsilylpropane, in which the vinylsilane acts as a silylating agent and hydrogen acceptor to form a disiloxane bond with the emission of ethylene. The easily removable trimethylsilyl group opens the possibility for further modification of the obtained silica surface. The functionalized CPG beads may be used for the synthesis of a DNA fragment permanently linked to a support. The results reveal that: (i) the siloxane bond is stable during the synthesis and removal of the protecting groups under basic conditions, and (ii) the oligonucleotide growing freely on this support is able to form secondary structures in the hybridization process.

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4

G. Hreczycho et al. / Tetrahedron Letters xxx (2013) xxx-xxx

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- Synthesis of 1-trimethylsiloxy-3-dimethylvinylsilylpropane (4): A 50 mL, twonecked, round-bottomed flask equipped with a magnetic bar and an argon bubbling tube was evacuated and flushed with argon. Then 5 g (0.086 mol) of allyl alcohol was added followed by a dropwise addition of 7.3 g (0.045 mol) of HMDS. The mixture was stirred at room temperature for 1 h. Next, the product was isolated by "bulb to bulb" distillation to give 10.75 g (0.0825 mol) of allyloxytrimethylsilane in 95.87% yield as a colorless liquid. A mixture consisting of 10.75 g (0.0825 mol) of allyloxytrimethylsilane, 8.55 g (0.09 mol) of chlorodimethylsilane, and 50 ml of CH₂Cl₂ was placed in a twonecked, 100-mL flask equipped with a magnetic bar and a reflux condenser. Next, 1×10^{-4} equiv of Karstedt's catalyst was added and the mixture was heated under a flow of argon for 4 h at 35 °C with stirring. The solvent was removed and the product isolated by 'bulb-to bulb' distillation to give 16.88 g (0.075 mol) of 1-trimethylsiloxy-3-chlorodimethylsilylpropane in 91% yield as a colorless liquid. Finally, 16.88 g (0.075 mol) of 1-trimethylsiloxy-3chlorodimethylsilylpropane and 50 ml of THF were added to a two-necked, 250-mL flask equipped with a magnetic bar and a reflux condenser. At 0 °C, 78.75 ml (1.05 equiv) of vinylmagnesium bromide (1 M in THF) was added dropwise. The mixture was stirred for 1 h, then the solvent was removed and the product was isolated by "bulb-to bulb" distillation to give 13.8 g (0.064 mol) of 1-trimethylsiloxy-3-dimethylvinylsilylpropane in 85% yield as a colorless liquid. ¹H NMR (C6D6) δ (ppm): 0.06 (s, 6H, SiCH₃), 0.14 (s, 9H, OSiCH₃), 1.52-1.60 (m, 2H, SiCH₂), 1.62-1.66 (m, 2H, SiCH₂CH₂), 3.53 (t, 2H, SiOCH₂), 5.68 (dd, J = 20.1, 4.0 Hz, 1H), 5.94 (dd, J = 14.7, 4.0 Hz, 1H), 6.16 (dd, I = 20.1, 14.7 Hz, 1H). ¹³C NMR (C6D6) δ (ppm): -3.5 (SiCH₃), -0.3 (OSiCH₃), 11.4 (SiCH₂), 27.6 (SiCH₂CH₂), 65.5 (SiOCH₂), 131.8 (SiCH=CH₂), 139.1

(SiCH=CH₂). MS (EI) *m*/*z* (rel int): 201 (15), 189 (40), 159 (100), 147 (85), 131 (50), 99 (25), 85 (85), 73 (40), 59 (50).

- 18. Representative procedure for the catalytic immobilization reactions (Table 1, entries 1–3): A mixture consisting of 0.15–0.3 g (0.7–1.4 mmol) of **4**, 1 g of amorphous silica gel [controlled pore glass (Cormay Porous Glass, Poland), dp = 125–160 μ m, Vp = 1.06 cm³ g⁻¹, *D* = 94 nm], 10–20 mg (0.014–0.028 mmol) of RuHCl(CO)(PCy₃)₂ and 10 ml of toluene were placed in a 25 ml Schlenk bomb flask fitted with a plug valve and heated at 110 °C for 4–17 h. After cooling to room temperature, the mixture was filtered and washed with toluene and dried under reduced pressure for 12 h to give 1.006–1.01 g of the grafted silica.
- 19. Determination of the level of the surface coverage by (loading) hydroxyl groups: A small portion of the support (about 8 mg) was weighed precisely. The support was then washed with 0.1% HCl (2 mL) and MeCN (10 ml). Subsequently, the reaction was conducted with 5'DMT 3'thymidine phosphoramidite as a weak acid in the presence of 5-benzylmercaptotetrazole (BTT) sequentially using the procedures of capping, oxidation, and deblocking. The synthetic cycle was repeated three times and deblocking solution was collected. The collected solution was diluted to 10 mL with CH₂Cl₂ with the addition of a small portion of toluenesulfonic acid and the absorption was measured. The trityl cation absorption measurement was used to calculate the amount of free hydroxyl groups capable of reacting with the nucleoside using the following formula:

 $Z = A^* V / \varepsilon_{\text{DMT}} + m$

where: Z – is the level of coverage (loading) of the surface hydroxyl groups, A – the measured absorbance, V – the sample volume, ε_{DMT} – the molar extinction coefficient (which for the DMT is 70), m – the mass of support.

20. Immobilization on the vinylsilane modified CPG particles.

In order to verify the efficiency of the vinylsilane modified to a solid support, we synthesized an oligomer (ODN-1) on it. The synthesis of oligonucleotides was performed by the phosphoramidite approach. The presence of ODN-1 on the support was confirmed by hybridization with complementary labeled nucleic acid (ODN-2) with a fluorescent marker. The outcome of hybridization was visualized by a fluorescence microscope (Fig. 1).

ODN-1 CGT CAC TAG AGG AAC GAG TAT $\overrightarrow{\text{GCC}}$ $\overrightarrow{\text{AGT}}$ CGT TAT GGT CCC TTG TAT CAA CT.

ODN-2-CTG GCA TAC TCG TTC CTC TAG TGA CG-Fluorescein.