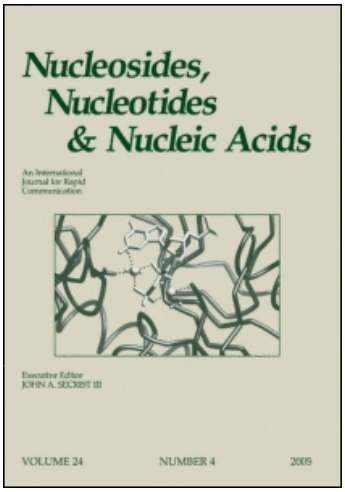

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STUDIES ON THE ATTACHMENT OF DNA TO SILICA-COATED NANOPARTICLES THROUGH A DIELS-ALDER REACTION

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▫ *A new method has been investigated for the functionalization of gold nanoparticles with DNA. Silica-coated nanoparticles functionalized with a maleimide have been prepared. These particles are designed to react with modified DNA containing a diene functionality at one end of the molecule. The result would be the formation of a more stable attachment of the DNA to the particle through a Diels-Alder reaction. This covalent attachment would not be susceptible to ligand exchanges, which are known to occur in the conventional DNA functionalization of gold nanoparticles.*

INTRODUCTION

Nucleic acid–coated gold nanoparticles are finding an increasing number of diverse applications. In particular, the ability of the DNA to direct nanoparticle assembly through hybridization can be used as the basis of a simple sensor system in biomedical diagnostics[1] and as a means to form supramolecular structures.[2,3]

The conventional method for preparing DNA-functionalized gold nanoparticles uses DNA chemically derived with either a 3′- or 5′- terminal thiol group and results in the DNA bound to the surface of the particle through an S-Au bond.[4] Particle stabilility increases with increasing DNA coverage, but hybridisation efficiency decreases with increasing DNA coverage. This method requires the DNA to be purified and isolated by conventional techniques prior to attachment to the gold. In addition the thiol bond to the gold surface is not sufficient to accomplish a permanent linkage, and in the presence of other thiols (e.g., DTT) equilibrium is

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established with dynamic ligand exchange. We have started to examine a more direct method for the preparation of DNA functionalized gold particles in which the DNA is bound to the surface through a permanent carbon–carbon bond. The strategy involves attaching silica-coated gold particles to the 5′-end of DNA as the final step in the automated synthesis using a Diels-Alder reaction. This procedure should result in very stable gold nanoparticles that have a permanent attachment to the DNA and can be isolated simply by centrifugation of the particles following deprotection of the DNA.

RESULTS AND DISCUSSION

Gold nanoparticles are obtained by mixing an aqueous solution of a gold salt with a solution of a stabilizing agent, citrate, which leads to reduction of the metal and at the same time adsorbs to the gold surface, stabilizing the colloid through electrostatic stabilizing forces. More stable nanoparticles are obtained by covering the surface of gold with a silica shell.[5] The prevention of flocculation is more effective than in the common stabilization with citrate as the electrostatic stabilizing interactions are replaced by covalent bonds. The resulting silica-coated nanoparticles react easily with alkoxysilane derivatives through condensation of terminal OH groups. In our case, a ligand containing a free maleimide has been attached to the silica-coated nanoparticles. The gold nanoparticles have been covered in a first step with 3-aminopropyltrimethoxysilane (APS) and a buffered solution of sodium silicate. The molecules of APS are linked to the silica nanoparticles through the amine group and the silane groups directed away from the particle surface. There are variants of this method, for example, based in the use of sulphur instead nitrogen as binding atom.[6] This generates a shell about 3 nm thick. In a second step, condensation of the silica groups was performed through base-catalysed hydrolysis and condensation of tetraethylorthosilicate (TEOS) in ethanol. With this procedure a net around the particle is generated that avoids any ligand exchange. The thicker the shell, the more homogenous in size and shape the nanoparticles are. In this approach, nanoparticles with 60 nm of total diameter have been prepared. After eliminating the excess ammonia and TEOS by centrifugation, N-(propyltrimethoxysilane)maleimide was added in ethanol. The ligand was added in a ratio of 1:20 (nanoparticle: ligand). After refluxing for 45 minutes, the excess of the maleimide was eliminated by centrifugation. The presence of the maleimide can be detected by the appearance of two absorption bands at 1,730 cm^{-1} in the IR-spectrum due to the carbonyl groups (Figure 1).

The synthesis of two different maleimides has been studied: N-(propyltrimethoxysilane)maleimide, and N-(propyltriethoxysilane)maleimide. The synthesis of these maleimide derivatives presented several difficulties due to the presence of the silane groups. One of the most conventional strategies to synthesise maleimides is the condensation of an anhydride and an amine at high temperature.[7] Thus, the dienophile was synthesised in two steps (see Figure 2). In the first step,

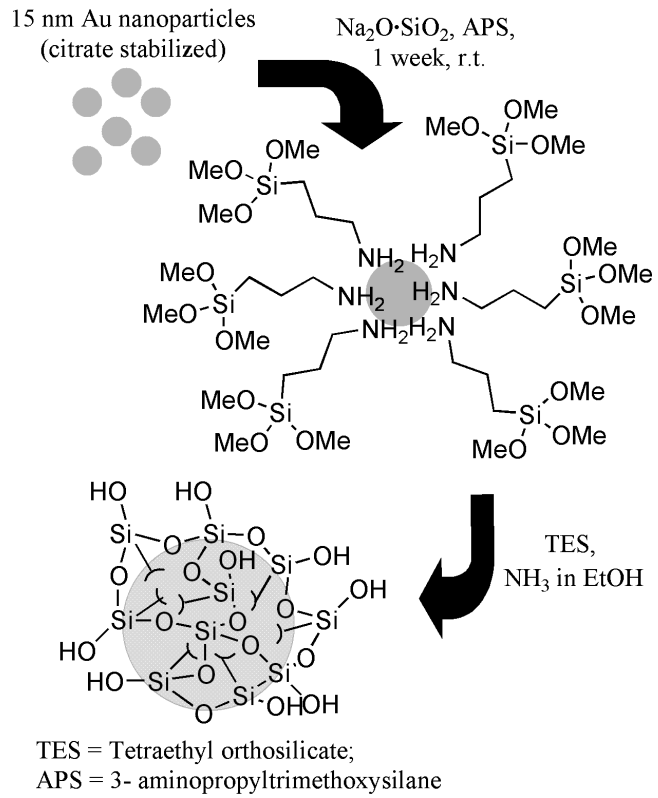

FIGURE 1 Synthesis of silica-coated gold nanoparticles.

N-propyltriethoxysilane is added to maleic anhydride in dichloromethane to obtain N-(propyltriethoxisilane)maleamic acid as an intermediate. The reaction took place readily (1 h) and the product was isolated as white crystals simply by removing the solvent.

The cyclization of the maleamic acids to the maleimides has been investigated by two different methods. In the first one, the N-(propyltriethoxisilane)maleamic acid was treated with triethylamine and trimethylsilylchloride (TMSCl) in DCM over night. Poor results were obtained with this method and very low yields of the maleimide were obtained (10–17% yield after distillation). In a second method, the maleamic acid was treated with zinc chloride and hexamethaldisalazane at 80°C.[8] Under these conditions the N-(propyltriethoxisilane)maleimide was obtained in 40% yield and its structure confirmed by ^{1}H NMR.

N-(propyltrimethoxysilane)maleamic acid was synthesised from N-propyltrimethoxysilane and maleic anhydride. The same features explained for the ethyl analogue were found. Both maleamic acids were obtained as white crystalline solids by simply removing the solvents. However, in some experiments N-(propyltrimethoxysilane)maleamic acid was obtained contaminated with the product of the

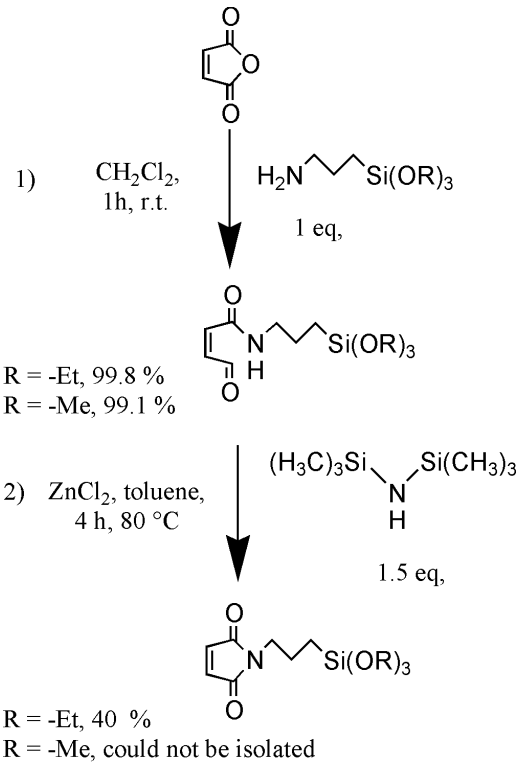

FIGURE 2 Synthesis of N-(propyltriethoxysilane)maleimide and N-(propyltrimethoxysilane)maleimide.

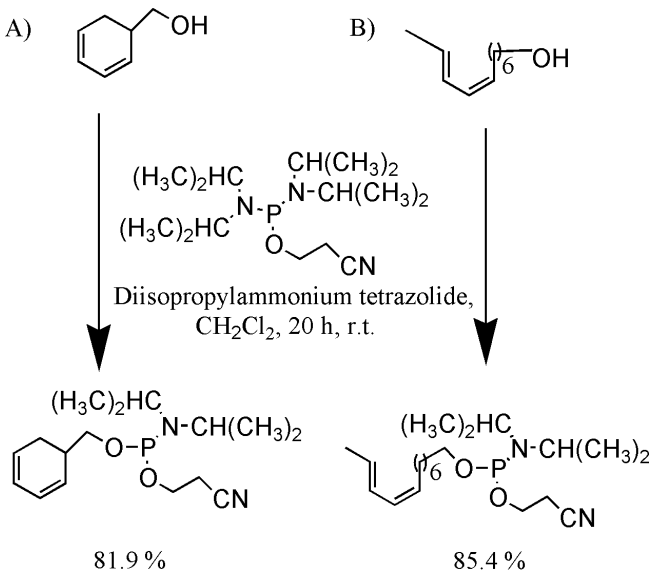

FIGURE 3 Synthesis of diene-derivatized phosphoramidites. A) Derived from 1,3-hexacyclohexene. B) Derived from 8,10-dodecadien-1-ol.

polymerization of the silane groups and the prevention of the polymerization of the methoxysilane groups has shown to be difficult. The cyclization of N-(propyltrimethoxysilane)maleamic acid has led to a mixture of polymers following the two methods described for the ethyl analogue and the isolation of the N-(propyltrimethoxysilane)maleimide was unsuccessful (Figure 3).

As a reactive partner for the maleimide in the Diels-Alder, an oligonucleotide derivatized with a diene is required. In order to attach a diene to the 5′- end we have prepared a DNA strand of two different phosphoramidites with 8,10-dodecadien-1-ol (commercially available) and 1,3-hexacyclohexene.[9] The Diels-Alder reaction between the diene-derivatized oligonuclotide and the maleimide-functionalized nanoparticle is still under investigation. DNA strands that had not reacted with the silica-coated gold nanoparticles will stay in the supernatant, while the DNA-gold aggregates will precipitate.

CONCLUSIONS

In this approach, we have functionalized with a maleimide more stable silica-coated gold nanoparticles that can be used in presence of another ligands in solution without ligand exchanges. Modified DNA containing a diene at the 5′- end of the molecule has been prepared for reaction with the particles through a Diels-Alder reaction leading to a permanent C–C bonding. This could be done before deprotection and cleavage from the solid support of the DNA and would allow the separation of the DNA functionalized particles from the free DNA strands just by simple centrifugation.

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