Pergamon

Tetrahedron Letters 42 (2001) 2197–2200

TETRAHEDRON LETTERS

Synthesis of a benzotriazole phosphoramidite for attachment of oligonucleotides to metal surfaces

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Received 7 April 2000; revised 10 January 2001; accepted 17 January 2001

Abstract—A method for the addition of a benzotriazole moiety to the 5′-terminus of an oligonucleotide via phosphoramidite chemistry has been developed. Use of a monomethoxytrityl protecting group on the benzotriazole allowed fast on-column detritylation purification by reverse-phase HPLC. Surface enhanced Raman scattering (SERS) of the modified oligonucleotides was obtained from silver colloid.

Oligonucleotides containing a metal complexing group are of interest for the attachment of oligonucleotides to metal surfaces. Traditionally oligonucleotides have been attached to gold surfaces by a thiol linker via the 5′-terminus of the oligonucleotides.[1,2] Recently Mirkin and co-workers have reported extensive use of gold colloid coated in oligonucleotides for DNA sequence analysis and also fabrication of nano-structures.[3–6] In this letter we report an alternative linker that allows attachment of oligonucleotides to other metal surfaces such as silver and copper. This allows oligonucleotide modified metal surfaces to be constructed, but is also of particular interest for surface enhanced Raman scattering (SERS). For SERS to occur the target molecule must be adsorbed onto a suitable metal surface.[7,8] The metal surface used in our spectroscopic studies is silver colloid and as such requires a suitable complexing agent to form an irreversible complex between the complexing agent and the metal. A suitable complexing agent for this purpose is benzotriazole.[9] Benzotriazole is known to form a polymeric layer with silver and copper metals and is commonly used as an anti-corrosion agent to prevent tarnishing.[10,11] As part of our research we have developed a convenient route for the synthesis of a benzotriazole phosphoramidite and used the monomer to prepare 5′-benzotriazole modified oligonucleotides. This allowed oligonucleotides to complex to metal surfaces via the 5′-terminus unlike unmodified oligonucleotides which do not complex and hence do not produce SERS.

Keywords: benzotriazole; HPLC; nucleic acids; phosphoramidites; solid-phase synthesis; SERS.

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Previously we have added a benzotriazole moiety to an amino linker at the 5′-end of an oligonucleotide but this takes time, requires a manual-coupling step and is not as high yielding. In our preferred approach we synthesised a benzotriazole phosphoramidite that contained an alkyl spacer between the benzotriazole and the phosphorus. The spacer ensured that the action of the benzotriazole was not affected by the presence of the larger oligonucleotide.

The starting material for the synthesis of the phosphoramidite was benzotriazole-5-carboxylic acid, which is commercially available (Fig. 1). In a separate reaction 6-aminohexanol was protected with a *tert*-butyldiphenylsilyl group to yield 6-*tert*-diphenyl-silanyloxyhexylamine (**1**). The amine was then reacted with the carboxylic acid to form an amide linkage (**2**) using carbonyldiimidazole as the activating agent. Carbonyldiimidazole was chosen as it has been used before with benzotriazole carboxylic acid and allows amide formation at the acid function without the need for protection of the triazole ring system.[12] However, the benzotriazole ring proton still required protection prior to the formation of the phosphoramidite. Thus, the monomethoxytrityl group was chosen for this purpose as it is compatible with solid-phase synthesis of oligonucleotides and allows trityl on purification. The benzotriazole linker was protected using monomethoxytrityl chloride in pyridine with a catalytic amount of dimethylaminopyridine. This yielded the fully protected compound *N* - [4 - methoxytrityl) - benzotriazoyl - 5 - carboxylic acid - (6 - tertbutyl - diphenyl - silanyloxy - hexyl)-amide (**3**) in 54% yield. A benzoyl protecting group was also tested but subsequent phosphitylation resulted in a poor yield hence the use of the more favoured

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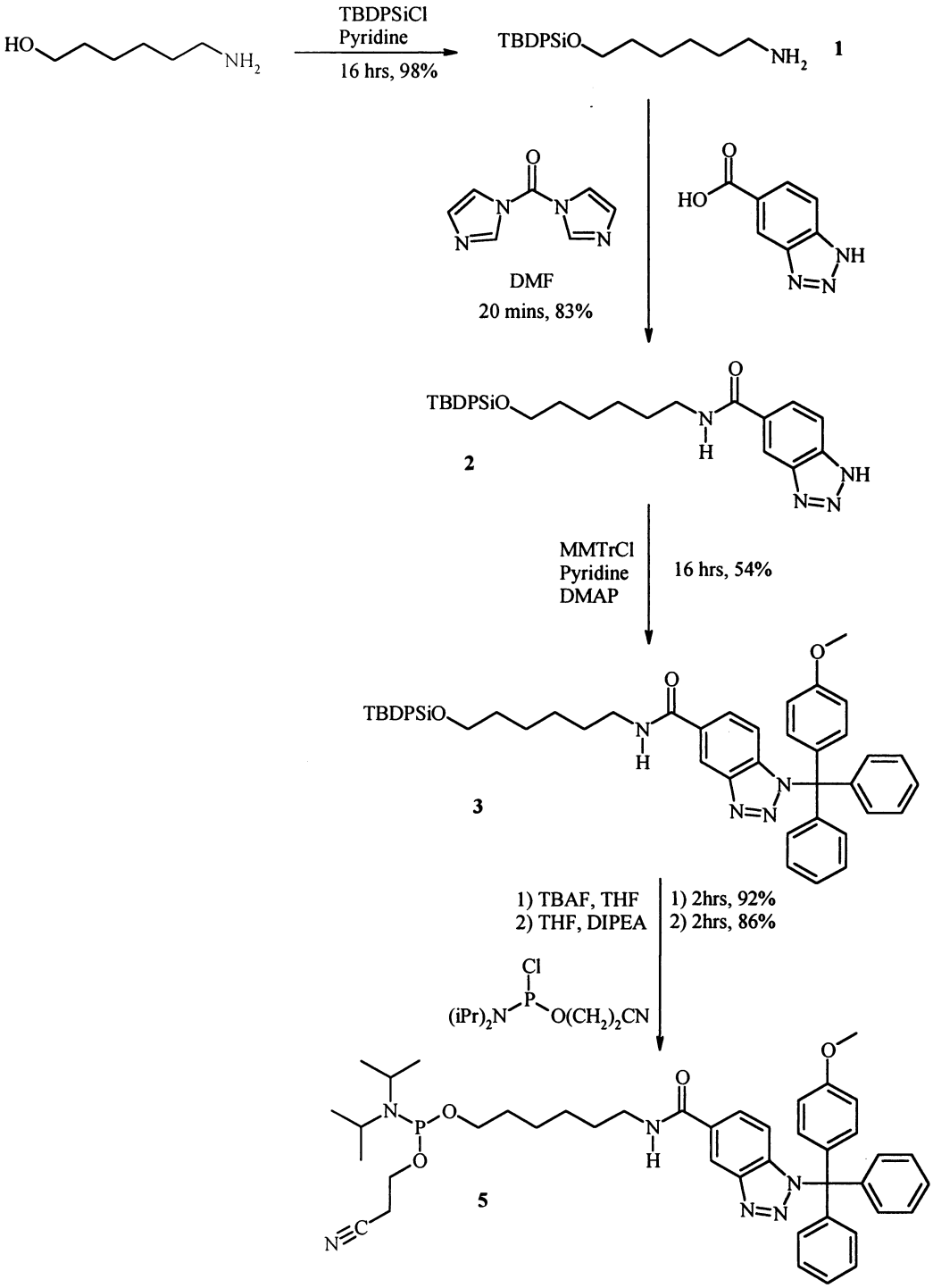

Figure 1. Synthesis of the benzotriazole phosphoramidite.

monomethoxytrityl group. *N*-Acylated benzotriazole can act as an acylating agent itself as the benzotriazole is comparable to a weak halide in leaving group nature in this case.

Removal of the silyl-protecting group was accomplished by tetrabutylammonium fluoride in THF followed by treatment with DOWEX H^+ in 92% yield to produce the primary alcohol for phosphitylation (**4**). A standard phosphitylation was performed in THF using 2 - cyanoethyl - *N*,*N* - diisopropylchlorophosphoramidite and diisopropylethylamine to yield the product (**5**) in 86% after 2 hours. The purity of the phosphoramidite was confirmed by ^{31}P NMR (148.78 ppm) prior to dissolution in anhydrous THF and used in routine solid-phase oligonucleotide synthesis.

Two 12 mer sequences and one 30 mer were synthesised using fast deprotection monomers[13] and the benzotriazole monomer added at the 5′-terminus via an extended coupling cycle using a double delivery and 15 minute coupling time. Deprotection by ammonia at room tem-

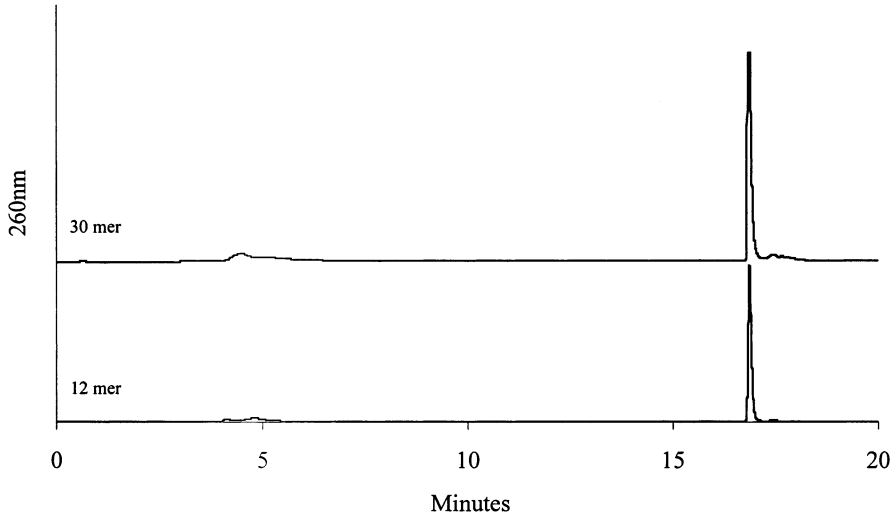

Figure 2. Analytical HPLC trace of on-column detritylation and purification for the two sequences at 260 nm. 12 mer 5′-BT TCT ATA TTC ATC and 30 mer 5′-BT GTA TCT ATA TTC ATC ATA GGA AAC ACC ATT.

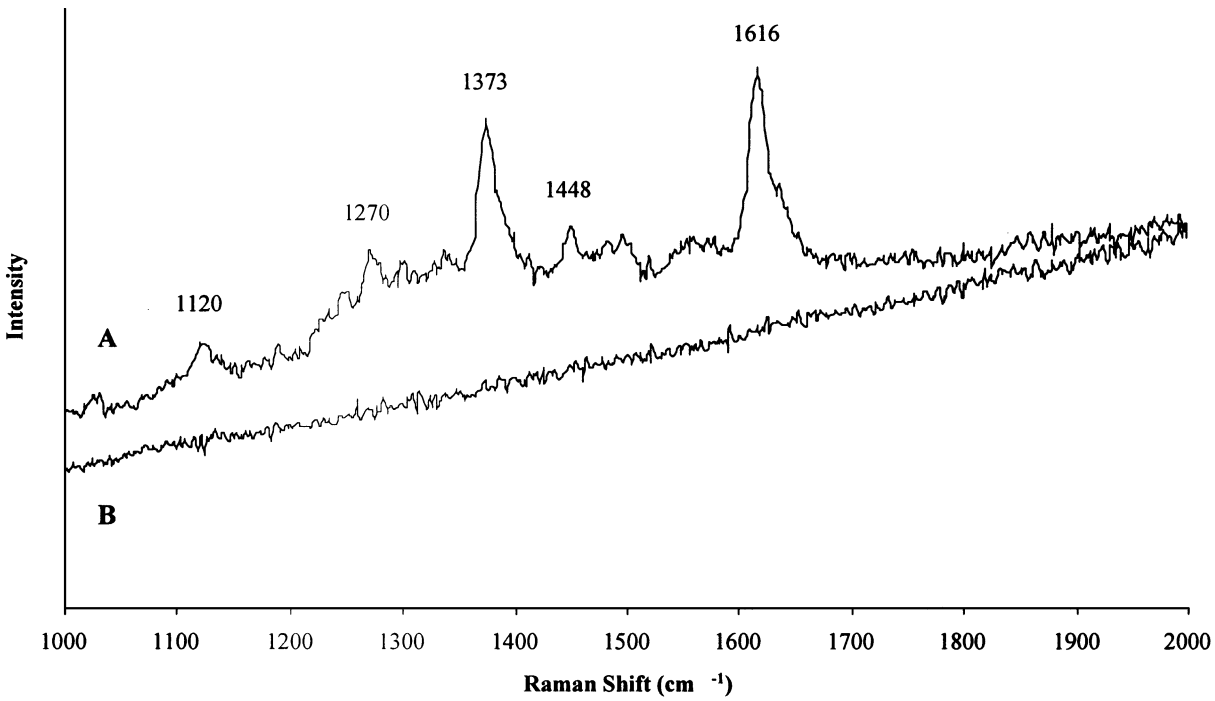

Figure 3. SERS spectra of benzotriazole modified oligonucleotide (A) and the control spectra of colloid with an unmodified oligonucleotide (B).

perature for 2 hours was followed by purification by reverse phase HPLC using a Poros oligo R3™ column and on column detritylation[14] (Fig. 2). The coupling efficiency was estimated as being between 80 and 90% by HPLC integration. The identity of both oligonucleotides was confirmed by electrospray MS.[15]

The benzotriazole oligonucleotides were then investigated for their ability to complex to a silver surface by examination of their SERS activity. SERS can only be seen for molecules on the surface of the silver colloid hence the oligonucleotide will only produce SERS if attached to the metal surface. The metal surface used was that of citrate reduced silver colloid, which has previously produced excellent SERS from benzotriazole azo dyes.[9] In this case the oligonucleotides were premixed with spermine (to neutralise the phosphate backbone[16]) and added to the silver colloid then left for 15 minutes to allow surface complexation. The colloidal suspension was concentrated by centrifugation and the supernatant removed before examination of the residue by SERS.[17] SERS signals were obtained from the benzotriazole oligonucleotides at a level of 5×10^{-8} mol, however, nothing could be seen from the control sample of colloid with DNA and spermine (Fig. 3). The bands arising at 1373 and 1616 cm^{-1} were assigned to the triazole ring and the phenyl ring systems of the benzotriazole, respectively, with the band at 1270 cm^{-1}

arising from the phenyl-triazole ring system. The signals were similar to those obtained for the benzotriazole carboxylic acid starting material as expected.

In conclusion, we have developed a simple route for the synthesis of a benzotriazole phosphoramidite and used it to incorporate benzotriazole into oligonucleotides via routine solid-phase synthesis. This has produced oligonucleotides with specific metal complexing properties. The benzotriazole modified oligonucleotides were shown to produce SERS from silver colloid thus providing evidence of surface attachment. This method of oligonucleotide modification will be of use in anchoring DNA to a number of metal surfaces for use in both structural and analytical studies.

Acknowledgements

The authors wish to thank the BBSRC for the award of a David Phillips fellowship to D.G. and Astra Zeneca for funding R.B.

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14. Buffer A—50 mM NH_4OAc pH 10; Buffer B—50 mM NH_4OAc pH 10+5% MeCN; Buffer C—0.2% TFA; Buffer D—65% MeOH 35% H_2O. Method run on Biocad Sprint HPLC load, 3 min A, 3 min B, 3 min A, 4 min C, 3 min A, 4 min D at 5 ml/min.
15. Electrospray MS found 3971.0, calculated 3970.8.
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17. SERS signals were accumulated using a Renishaw 2000 Raman Microprobe instrument with 514.5 nm excitation. Spectra were accumulated three times for 30 seconds.