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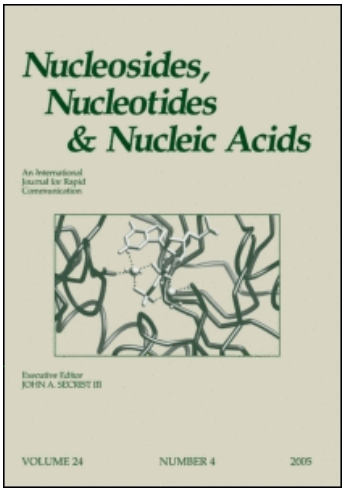

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SYNTHESIS OF MODIFIED BUILDING BLOCKS CONTAINING AMINO OR THIOL MOIETIES: APPLICATION OF MODIFIED OLIGODEOXYRIBONUCLEOTIDES

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ABSTRACT: Modified building blocks have been synthesised and used to prepare 5′-amino and 5′-mercapto-oligodeoxyribonucleotides. Subsequent labelling with fluorophores or metal cluster derivatives generates a range of very useful probes.

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The interest in modified oligonucleotides for solving a variety of problems in molecular biology continues to grow. As our contribution to this field we recently reported the synthesis of protected 5′-mercapto-2′,5′-dideoxyribonucleoside-3′-O-phosphoramidites[1] and the corresponding 5′-amino compounds.[2] These building blocks were then used in an automated DNA synthesiser to prepare 5′-mercapto and 5′-amino-oligodeoxyribonucleotides. Subsequent labelling of these modified oligonucleotides with a variety of fluorescent derivatives has been used to produce fluorescent primers for fully automated DNA sequencing without radioactivity.[3,4] However, for the preparation of labelled primers we now prefer to use a linker molecule at the 5′- end of the chemically synthesised oligodeoxyribonucleotide. For example, we use 6-trifluoroacetylaminohexyloxy, 2-cyanoethoxy, N,N-diisopropylaminophosphine or 3-triphenylmethylmercaptopropyloxy, 2-cyanoethoxy, N,N-diisopropylamino-phosphine to prepare oligodeoxyribonucleotides bearing a 5′-spacer arm terminating in an amino or mercapto moiety. In some cases the use of the N-monomethoxytrityl group has advantages[5] over trifluoroacetyl.

The most important use of these 5′-amino and 5′-mercapto-oligodeoxyribonucleotides is for the preparation of metal cluster derivatives, notably triosmium and tetrairidium cluster compounds, which can be used in electron microscopy work. We have prepared a tetrairidium cluster derivative of 5′-amino-d[CCGATATCGG] and this has been cocrystallised with EcoRV. The ability to prepare 5′-mercapto and 5′-amino-oligodeoxyribonucleotides enables us to perform coupling with iodoacetamides or maleimides in the case of the HS-moiety, and with active esters in the case of the amino

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moiety. In addition the 5′-mercapto-oligodeoxyribonucleotides can be reacted with mercurials giving derivatives useful for x-ray crystallography.

Most recently we have completed the synthesis of a 2′-deoxyuridine building block bearing a C-5 spacer arm terminating in a triphenylmethylmercapto group for incorporation into oligodeoxyribonucleotides that can be labelled with an undecagold cluster and also still be ^{32}P labelled. These gold cluster labelled oligonucleotides will then be used as probes in cryo-electron microscopy.

In order to synthesise the 5′-mercapto building blocks we developed the following procedures; full experiment details are given elsewhere:[1,2] Thymidine was reacted with methyltriphenoxyphosphonium iodide[6] to give 5′-iodo-5′-deoxythymidine in good yield. Displacement of iodide by sodium triphenylmethylmercaptide in DMF followed by phosphitylation of the 3′-OH gave the desired 5′-(S-triphenylmethyl)mercapto-5′-deoxythymidine-3′-O-(2-cyanoethyl N,N-diisopropylphosphoramidite). The corresponding dC and dG building blocks were obtained by iodination of 3′-O-levulinyl-N^4-benzoyl-2′-deoxycytidine and 3′-O-levulinyl-N^2-isobutyryl-2′-deoxyguanosine respectively, followed by displacement of iodide with TrSNa, removal of the levulinyl group with hydrazine and subsequent phosphitylation. In the case of the modified dA building block, since attempted iodination was expected to lead to N^3,5′-cyclonucleoside formation[6] a slightly different route was adopted. N^6-Pivaloyl-3′-O-levulinyl-2′-deoxyadenosine was synthesised and converted into its 5′-(4-nitrobenzenesulphonate) in good yield. Subsequent displacement of the sulphonate moiety by TrSNa, followed by delevulinylation and phosphitylation generated the desired modified building block. The trityl group was chosen as the S protecting group because of its lipophilicity and because its presence enables the ready purification of 5′-(S-trityl)mercapto-oligodeoxyribonucleotides by reversed phase h.p.l.c. This protecting group is relatively acid stable, however, it is readily cleaved by silver[7] or mercuric ions.[8]

Base protected 5′-(N-trifluoroacetyl)amino-2′,5′-dideoxyribonucleoside-3′-O-(2-cyanoethyl, N,N-diisopropylphosphoramidites) were in brief synthesised as follows. A phosphoramidite derivative of 5′-amino-5′-deoxythymidine was already known.[9] N^4-Benzoyl-2′-deoxycytidine was smoothly iodinated at C-5′ and the iodide was then displaced by sodium azide in DMF; Raney-nickel reduction and trifluoroacetylation of the thus generated 5′-amino group with S-ethyl trifluorothioacetate followed by phosphitylation of the 3′-OH gave the desired modified dC building block. In order to synthesise the 5′-amino modified dG and dA building blocks, 3′-O-t-butyldiphenylsilyl-N^2-isobutyryl-2′-deoxyguanosine and 3′-O-t-butyldiphenylsilyl-N^6-pivaloyl-2′-deoxy-adenosine were initially prepared. Iodination of the former compound and p-

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nitrobenzenesulphonylation of the latter gave intermediates which reacted cleanly with sodium azide in DMF. Reduction, trifluoroacetylation, desilyation and finally phosphitylation generated the desired modified dG and dA building blocks.

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