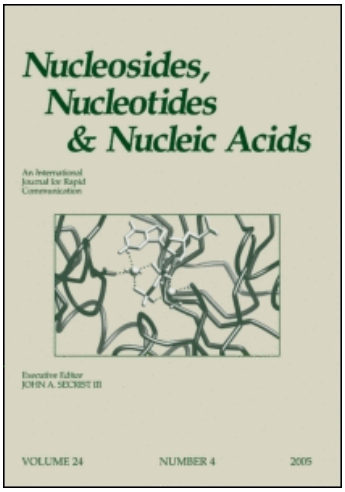

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New developments in the synthesis of oligonucleotide-peptide conjugates

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NEW DEVELOPMENTS IN THE SYNTHESIS OF OLIGONUCLEOTIDE-PEPTIDE CONJUGATES

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□ *The stability of oligodeoxynucleotides to trifluoroacetic acid is studied. Pyrimidine oligonucleotides were stable in the conditions used for the removal of* t*-butyl groups. Oligonucleotide-3'-peptide conjugates carrying pyrimidine oligonucleotides are prepared stepwise using peptide-supports and Fmoc,* t*-butyl strategy. Using this strategy we have prepared an oligonucleotide-peptide conjugate containing as peptide the leucine-rich fragment of FOS, a transcription factor involved in many important cellular processes. This conjugate has a long peptide sequence with a large number of trifunctional amino acids.*

Keywords Oligonucleotide-peptide conjugates; pyrimidines; *t*-butyl groups; leucine zipper

INTRODUCTION

The use of synthetic oligonucleotides to control gene expression has triggered the search for new oligonucleotide derivatives with improved therapeutic potential.[1] Oligonucleotide-peptide conjugates are chimeric molecules consisting of oligonucleotides covalently linked to peptides. As a result, synthetic oligonucleotides acquire some of the biological and/or biophysical properties of peptides.

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Two strategies can be followed to synthesize oligonucleotide-peptide conjugates.[2] In the postsynthetic conjugation approach, the two moieties are prepared independently and specific groups (such as thiols and maleimido) are specifically incorporated to link both molecules.[3] In the stepwise approach, oligonucleotide-peptide conjugates are prepared by stepwise addition of amino acids and phosphoramidites in solid phase on the same solid support.[4] In this case, the problem is the incompatibility of the standard schemes of protection for peptides and oligonucleotides. For example, at the end of the solid-phase peptide synthesis, a treatment with acid is usually required, which can provoke partial depurination of DNA.

Recently, our group has undertaken the preparation of conjugates consisting of double-stranded DNA (dsDNA) and selected fragments of biologically relevant transcription factors. Specifically we focused on the synthesis of conjugates between DNA and peptidic regions from the leucine zipper domain of FOS.[5] These constructs, which display a dual recognition surface provided by the presence of both the peptide and the DNA, were devised as potential specific receptors for selected FOS partners, such as the well known oncogenic transcription factor JUN. Hypothetically, the recognition would involve a specific interaction between the leucine-rich region of JUN and the hybrid, and an additional interaction between the oligonucleotide portion of the constructs and the basic region of JUN.[6] The synthesis of these conjugates is difficult due to the presence of long peptide sequences with large number of trifunctional amino acids. In any case we evaluated the viability of making this type of oligonucleotide-peptide conjugates using a stepwise Fmoc, *t*-butyl strategy.

RESULTS AND DISCUSSION

Stability of Oligodeoxynucleotides to Trifluoroacetic Acid

Trifluoroacetic acid (TFA) is a strong acid commonly used in the final deprotection step of peptide synthesis using Fmoc-amino acids. It is known that acidic solutions provoke depurination of purine nucleosides. On the other hand, depurination is slower in oligonucleotides and indeed several experiments on the use of TFA to remove Boc groups in the presence of oligodeoxynucleotides were described almost two decades ago.[7,8] In order to study the extension of this reaction we have treated 4 oligodeoxynucleotide sequences (T_9, T_4CT_4, T_4AT_4, and T_4GT_4) with 95% TFA aqueous solution. Two different treatments were studied. Treatment A: The oligonucleotide supports (polystyrene, LV200) obtained after the assembly of the oligonucleotide sequence still carrying the protecting groups were treated with 95% TFA aqueous solution. After 2 hours, the acidic solution was filtered out and the support was treated with concentrated

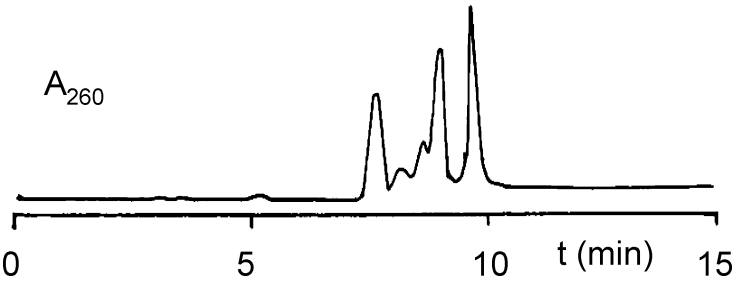


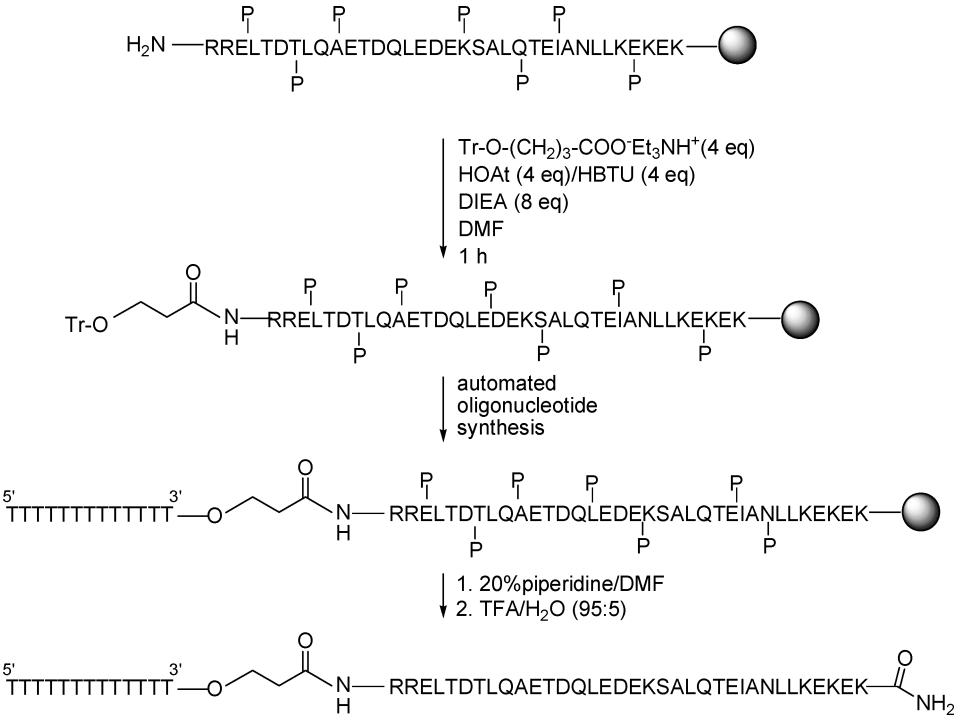
FIGURE 1 HPLC of the mixture resulting from the treatment of the support carrying T_4AT_4 followed by ammonia deprotection. The late-eluting peak is the remaining full-length oligonucleotide sequence T_4AT_4. HPLC conditions as follows. Nucleosil 120 C18 (250 x 8 mmm) column at a flow rate of 3 mL/minutes, detection 260 nm, gradient 0–50% B in 20 minutes, A: 5% CH_3CN in 100 mM triethylammonium acetate (TEAA) pH 7.0, B: 70% CH_3CN in 100 mM TEAA pH 7.0.

ammonia. The resulting solution was concentrated to dryness and analyzed by reversed-phase HPLC. Treatment B: Oligonucleotides were deprotected with ammonia and the resulting unprotected oligonucleotides were treated with 95% TFA aqueous solution for 2 hours. The resulting solutions were concentrated to dryness, treated with ammonia (to break apurinic sites) and analyzed by HPLC.

Pyrimidine oligonucleotides (T_9 and T_4CT_4), after treatment A, gave a single peak with the expected mass by MALDI MS, indicating stability of the oligonucleotides to the TFA treatment as it is expected due to the higher stability of the glycosidic bond of pyrimidine nucleosides. Exceptionally, treatment B on T_4CT_4 with TFA promoted a slight decomposition. Oligonucleotide sequence carrying adenine (T_4AT_4) gave two more peaks eluting before the peak of the initial oligonucleotide (Figure 1). This indicates loss of the adenine followed by hydrolysis of phosphate at the abasic site by ammonia. The extension of the depurination was around 80% in treatment A and 60% in treatment B. Oligonucleotide sequence carrying guanine (T_4GT_4) was stable to treatment A but unstable to treatment B (30% depurination). This is consistent with the higher stability of dG to depurination compared to dA. Depurination was more severe in treatment B probably because in solution reactions are faster and because of the additional time of exposure to acids during concentration of the solution.

Synthesis of Pyrimidine Oligonucleotide Conjugates Carrying the Leucine Zipper Domain of FOS

The stability of pyrimidine oligodeoxynucleotides to TFA suggest that pyrimidine oligonucleotide conjugates may be prepared by addition of the oligonucleotide sequence to the peptide support carrying the leucine zipper



SCHEME 1 Outline of the synthesis of oligonucleotide conjugate T_{13}-leucine zipper domain by the stepwise approach.

domain of FOS assembled with the standard Fmoc, *t*Bu-strategy. Scheme 1 outlines the synthetic approach. The peptide part is a peptide of 35 amino acids carrying 25 trifunctional amino acids, two of which are arginines. The oligonucleotide moiety is tridecathymidine.

The synthesis of the 35 amino acid peptide was carried out manually on a Rink-MBHA amide resin using Fmoc-protected amino acids and a symmetric anhydride strategy. In order to incorporate the oligonucleotide sequence to the peptide support, the amino terminal group was reacted with the O-4-trityl derivative of 4-hydroxybutanoic acid. This compound will generate the hydroxyl group which will be used to assemble the oligonucleotide sequence (Scheme 1). Figure 2 shows the HPLC analysis of the T_{13}-leucine zipper domain conjugate (Waters X-Terra column at a flow rate of 1 mL/min, detection 260 nm, gradient 15–80% B in 20 minutes, A: 5% CH_3CN in 100 mM TEAA pH 7.0, B: 70% CH_3CN in 100 mM TEAA pH 7.0). A major peak corresponding to the desired conjugate was observed and characterized by UV, PAGE, and MALDI MS. To the best of our knowledge these conjugates are among the largest and more complex oligonucleotide-peptide conjugates constructed to date by chemical synthesis, and in particular by solid-supported stepwise methods.

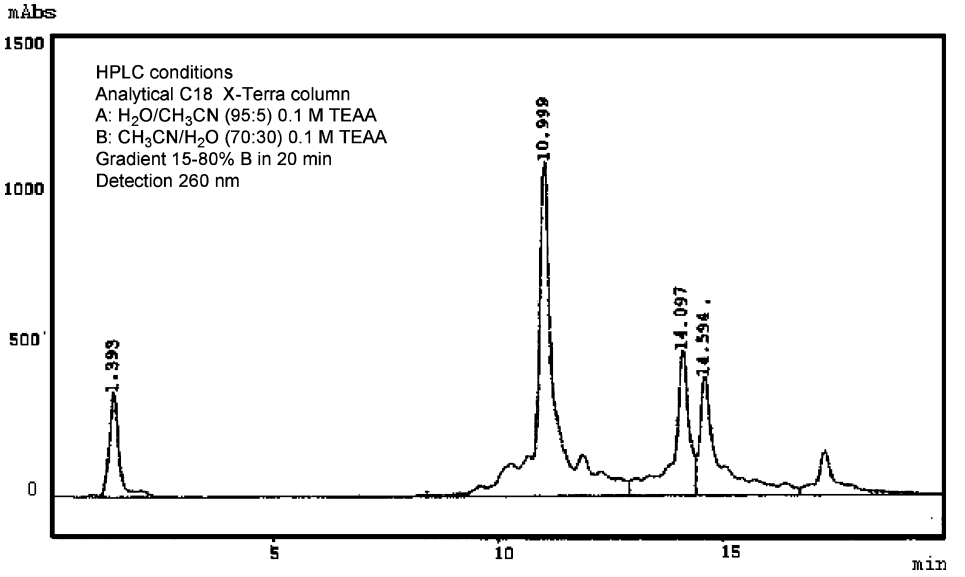


FIGURE 2 HPLC purification of oligonucleotide conjugate T_{13}-leucine zipper domain. The peak at 10.9 minutes is the expected oligonucleotide conjugate. HPLC conditions in the insert.

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