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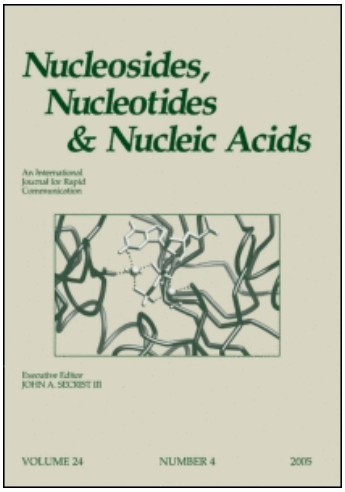

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A CONVENIENT METHOD FOR THE SYNTHESIS OF OLIGONUCLEOTIDE-CATIONIC PEPTIDE CONJUGATES

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▫ *A method was developed for the synthesis of oligonucleotide-cationic peptide conjugates in solution phase by disulfide bond formation. Precipitation was avoided by the easily removable triethylammonium trifluoroacetate (TEATFAc) salt which served at the same time as a buffer of the reaction mixture. The fast and high yielding disulfide bond formation was due to the Npys thio protecting and activating group of Cys. A solution of the free 5′-thiol modified oligonucleotide obtained from Poly-Pak™ purification was used for conjugation.*

Keywords Conjugation, Disulfide Bond Formation, Cationic Peptide, Penetration, Oligonucleotide

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INTRODUCTION

The precipitation caused by interaction of cationic peptides with anionic oligonucleotides can be avoided by adding salts,[1,2] urea,[3] or SDS[4] to the reaction mixture in high concentration. Unfortunately, removal of these needs multistep purification procedures leading to low overall yields. The effective intracellular delivery of antisense oligodeoxynucleosides (ODNs) can be achieved by their conjugation to cell-penetrating peptides, e.g. penetratin. While these peptides are highly cationic they neutralize the negative charges of ODNs hence precipitation occurs during the conjugation reaction.

RESULTS AND DISCUSSION

An easily removable ion pair forming reagent was required for the conjugation. Formation of a disulfide bond between Cys(Npys)penetratin and a 20-mer

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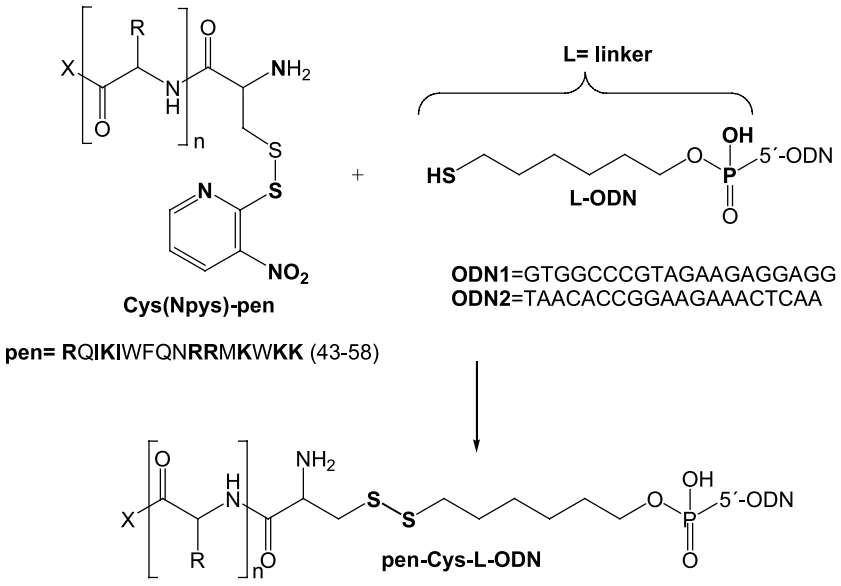
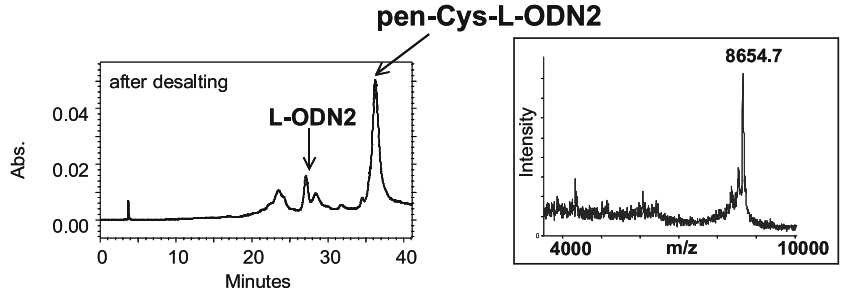


SCHEME 1 Conjugation reaction by disulfide bond formation between Cys(Npys)pen and ODN containing thio-linker at 5′-end.

oligonucleotide containing thio-linker at 5′-end[5] was chosen for testing of ion pair forming reagents (Scheme 1).

The formation of disulfide bond took place at pH 7 in a triethylammonium acetate (TEAAc) buffer. Unfortunately, the solution of the peptide was opalescent but replacing the acetate anion with trifluoroacetate (TFAc) helped to avoid precipitate formation. The free 5′-thiol modified oligonucleotide was used as a 20% aq. acetonitrile (ACN) solution obtained after a slightly modified Poly-Pak™ purification (the concentration of TFA was 4% instead of 2%), whereby trimethoxytrityl (TMTr) protecting group of the thiol group was removed quantitatively.

The conjugation was complete in 1 h with 3 *eq*. Cys(Npys)pen with no precipitation and symmetrical disulfide bond formation. Unfortunately, TEATFAc



SCHEME 2 HPLC and MALDI-TOF MS analysis of the conjugate pen-Cys-L-ODN2.

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cannot be removed with a simple lyophilization, therefore desalting was done by Poly-Pak™. The absorbance of solution after the reaction and desalting steps was 10.0 OD_{260} starting from a 9.7 OD_{260} value of conjugate L-ODN2. Further purification of the conjugate was done under routine HPLC conditions (RP-HPLC, 0–40% B, 40 min; A: 0.1 M TEAAc, B: A: ACN=2:8; see Scheme 2). MALDI-TOF MS analysis (positive linear mode) of L-ODN1 (calculated: 6469.2, measured: 6467.9; matrix: 3-hydroxypicolinic acid) and the conjugate pen-Cys-L-ODN2 (calculated: 8655.1, measured: 8654.7; matrix: 2′, 6′-dihydroxyacetophenone and diammonium hydrogen citrate) corroborated the above structures.

In conclusion, precipitation, one of the main problems of solution phase synthesis of cationic peptide oligonucleotide conjugates, can be avoided by using TEATFAc buffer, which is removable by several lyophilization or simple desalting step.

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