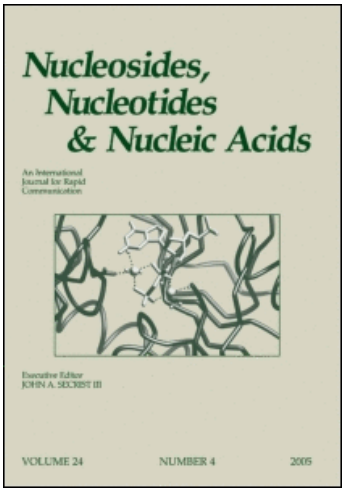

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A NOVEL APPROACH FOR THE SOLID PHASE SYNTHESIS OF DNA-PEPTIDE CONJUGATES

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A NOVEL APPROACH FOR THE SOLID PHASE SYNTHESIS OF DNA-PEPTIDE CONJUGATES

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ABSTRACT

The strategy of this study involves automated synthesis of oligonucleotides on a CPG support using standard cyanoethyl phosphoramidite chemistry followed by covalent linkage to peptide fragments bearing a free terminal α-amino group and residues with protected side chains. Conjugation was formed through an alkyldiisocyanate linker. Conjugates wereisolated by cleavage from the solid support and deprotection in one step.

INTRODUCTION

Genetic medicines such as antisense or antigene oligonucleotides, ribozymes and decoy RNAs have been attracting special interest from a medicinal and a biological aspect. As well as chemical modifications (1,2) on phosphodiester backbones, nucleobases, and/or sugar moieties, conjugation of oligonucleotides with functional peptides (3) is an alternative and fascinating way to improve the properties of native antisense and triple-helix forming oligonucleotides, for example, enhanced membrane permeability, improved stability against cellular nucleases and increased affinity and specificity.

Many reports have been ever made on the studies of solid phase syntheses of DNA conjugate molecules. However, most of them have limitations for their practical applications especially to DNA-peptide conjugates due to

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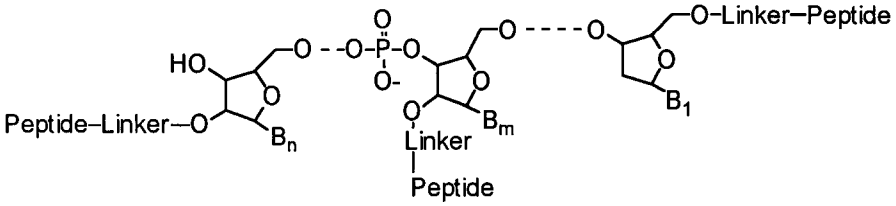


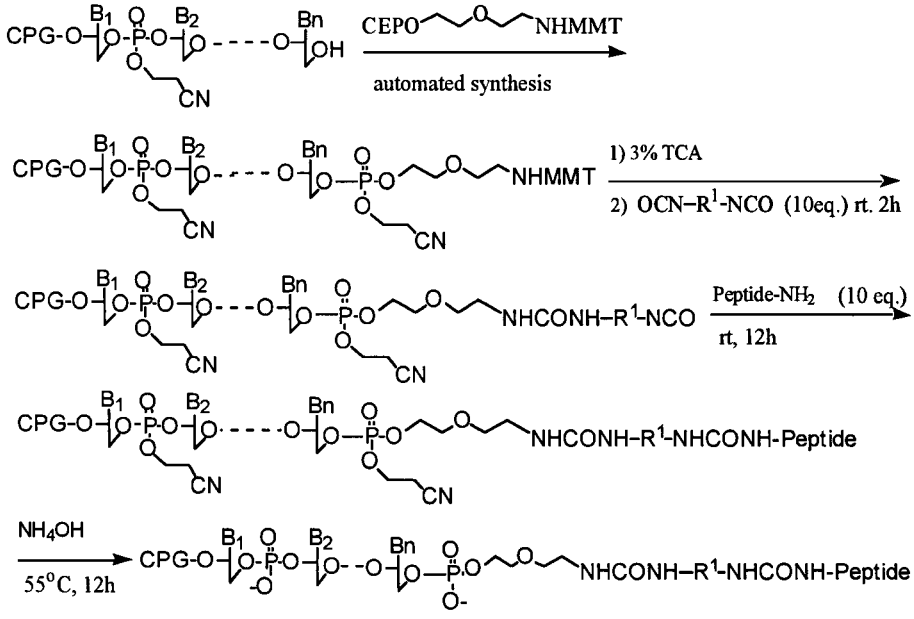
Figure 1. A Covalent Conjugate of DNA and Peptide.

different standard protocols between solid phase DNA synthesis and peptide synthesis.

The present study describes a novel and universal method for the solid phase synthesis of covalent DNA-peptide conjugates (Fig. 1) using fragment condensation strategy.

RESULTS AND DISCUSSIONS

Conjugate 1; 3′-GCTAGAGAGAGAGAAAAATCG-5′-$OPO_3(CH_2)_2O(CH_2)_2$-NHCO-NH-$(CH_2)_6$-NHCONH-bAla-$(LAKL)_3$-OH was synthesized as shown in Scheme 1.



Conjugate **1**;
3'-GCTAGAGAGAGAGAAAAATCG-5'-$OPO_3(CH_2)_2O(CH_2)_2NHCONH(CH_2)_6$-NH-CONH-βAla-$(LAKL)_3$-OH.
Yield. 24 % after HPLC purification.

Scheme 1. Preparation of Conjugate **1** Using 5′-Amino Modifier.

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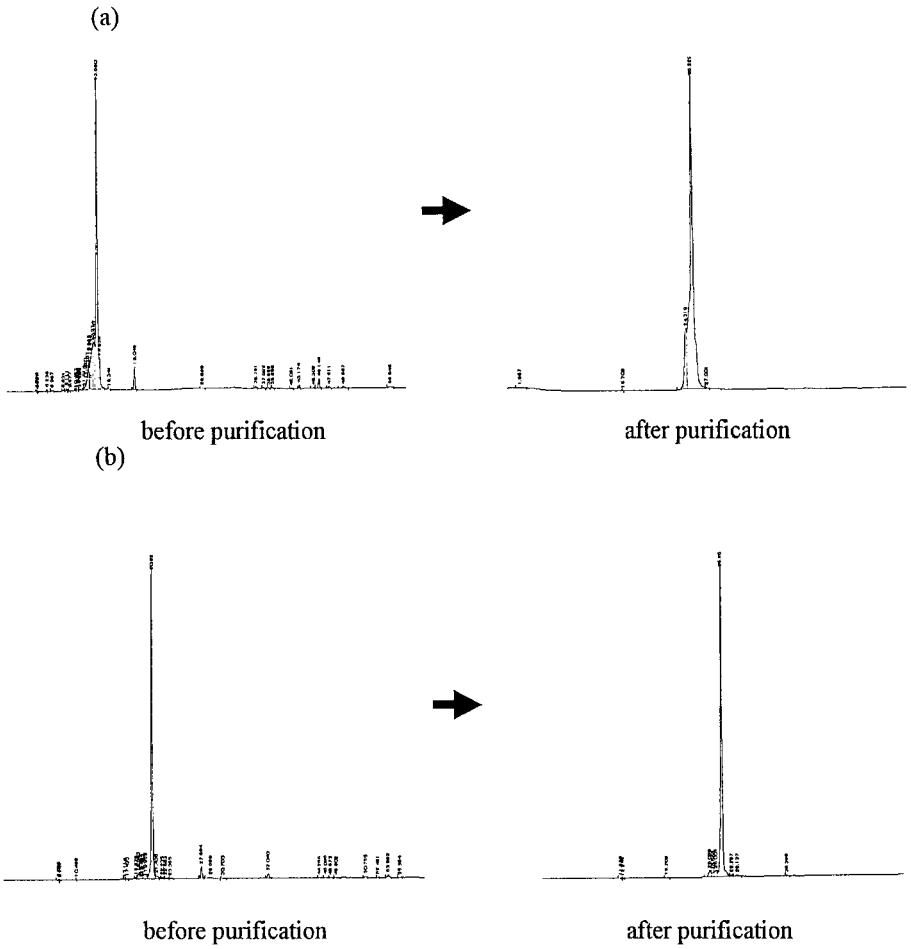

Figure 2. HPLC Profile of Purification of (a) Conjugate **1** and (b) Conjugate **2**. Conditions: ODS(C-18), monitored at 260 nm. linear gradient from 10 to 100% of buffer B in 60 min. at a flow rate of 0.3 ml/min. buffer A; 100 mM triethylammonium acetate, buffer B; 100 mM triethylammonium acetate in 70% CH_3CN.

DNA fragment assembled on CPG by using standard protocols were modified with 5′-amino modifier at 5′-terminus. After removal of MMT from terminal amino group with TCA, reaction with 1,6-hexamethylenediisocyanate at room temperature for 2 h afforded DNA fragment having an isocyanate group at the terminus, which was readily reacted with peptide fragment having free α-amino group and protected side chain residues (4). After deprotection with ammonium hydroxide at 55°C and purification by RPHPLC gave conjugate 1 in 24% yield (Fig. 2 (a)).

Conjugate 2; 3′-TCTCTCTCTCTTTTT-5′-OCONH-$(CH_2)_2$-NHCONH-$(CH_2)_6$-NH-CONH-$(LRAL)_3$-OH was prepared as shown Scheme 2.

Hydroxyl group at 5′-terminus of the oligonucleotide fragment assembled on CPG support was reacted with CDI (5), ethylene diamine, 1,6-hexamethylenediisocyanate, and then peptide fragment having free α-amino group and

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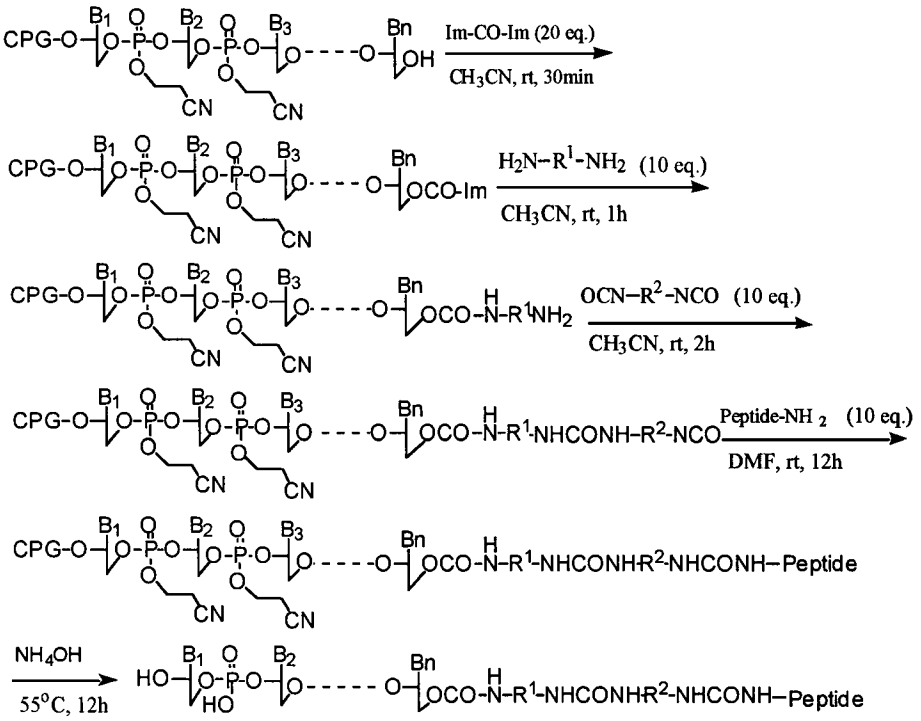

Scheme 2. Preparation of Conjugate **2** Using CDI and Alkyldiamine.

protected side chain residues. After deprotection with ammonium hydroxide at 55°C and purification by RPHPLC gave conjugate **1** in **19**% yield. (Fig. 2 (b)).

The present study can provide a practical and universal method for the solid phase synthesis of DNA-peptide conjugates at 3′ and 5′-end as well as in the middle of the nucleotides. Studies on hybridization and biological properties of thus synthesized conjugates are now in progress in our laboratory.

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