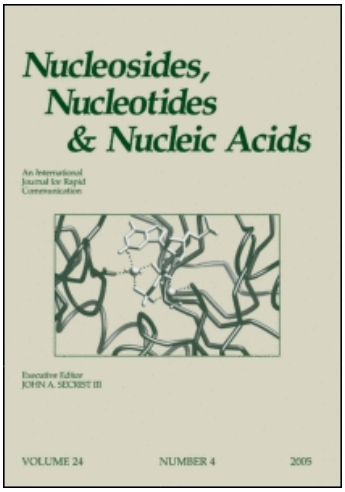

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Total Stepwise Solid-Phase Peptide-Oligonucleotide Conjugate Synthesis on Macroporous Polystyrene

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Total Stepwise Solid-Phase Peptide-Oligonucleotide Conjugate Synthesis on Macroporous Polystyrene

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ABSTRACT

An efficient total stepwise solid-phase synthesis of oligonucleotide-peptide conjugates on a macroporous polystyrene is described. Extending our homoserine linker approach, we prepared a range of fluorescein-labelled conjugates containing one of two different peptides together with oligonucleotides containing 2′-deoxynucleoside or 2′-*O*-methylribonucleoside phosphodiesters, or gapmers containing 2′-deoxyphosphorothioate sequences flanked by 2′-*O*-methyl wings.

Key Words: Peptide; Oligonucleotide; Conjugate; Solid phase synthesis.

Use of oligonucleotides as gene regulation agents has been hampered by poor cellular uptake. One solution is to conjugate the oligonucleotide to a peptide that possesses cell penetration properties to facilitate cellular delivery.[1,2] We described recently a novel method of synthesis of oligonucleotide 3′-conjugates using an ω-aminoalkyl succinate/L-homoserine linker.[3,4] Now we report modification of this

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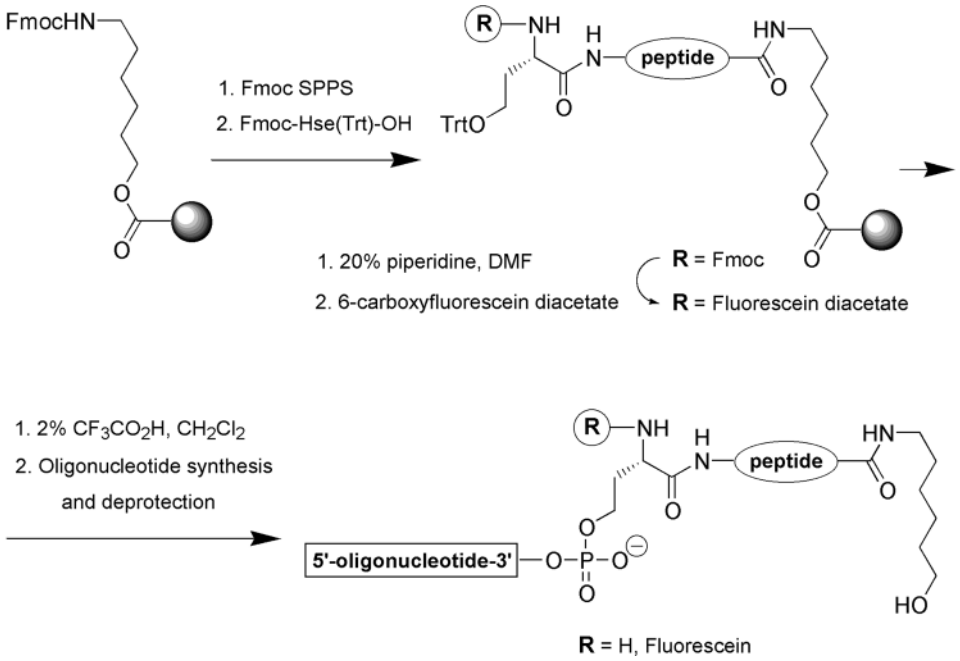

Scheme 1.

method towards the total stepwise solid-phase synthesis of peptides conjugated to antisense oligonucleotides and their 2′-*O*-methyl analogues (see Sch. 1).[5]

As antisense models (Table 1) we have used a 15-mer 2′-deoxyoligonucleotide and 12-mer and 16-mer 2′-*O*-methyloligoribonucleotide (OMe) sequences, as well as gapmers consisting of a 2′-deoxyphosphorothioate 8-mer sequence flanked by OMe 5-mer wings. We have chosen two peptides (Table 2) that were reported to have membrane activity: a 15-mer Kaposi fibroblast growth factor peptide (K-FGF) (**1**), and a 21-mer short version of Transportan (**2**). While the first peptide does not require side-chain protection, Transportan includes asparagine and tyrosine as well

Table 1. Sequences of antisense oligonucleotides.[a]

cucccaggcuca	**(I)**
CTCCCAGGCTCAAAT	**(II)**
GCTCCCAGGCTCAAA	**(III)**
AGCTCCCAGGCTCAA	**(IV)**
ugugc**TATTCTGT**gaauu	**(V)**
uaagc**TGTTCTAT**guguu	**(VI)**
cucccaggcucagauc	**(VII)**

[a]2′-Deoxynucleotides are in capitals, 2′-deoxy phosphorothioates in bold capitals, and 2′-*O*-methyls in lower case.

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Table 2. Sequences of cell-penetrating peptides.[b]

AVALLPAVLLALLAP	(**1**)
AGYLLGK(Ac)INLKALAALAKKIL	(**2**)

[b]All peptides are *C*-terminal 6-hydroxyhexylamides and contain L-homoserine residue at the *N*-terminus, optionally α-*N*-acylated with 6-carboxyfluorescein.

as lysines, the ε–amino groups of which are protected by trifluoroacetylation.[3] We opted for the acid-labile 2-chlorotrityl group for the tyrosine residue. The asparagine residue was incorporated via Fmoc-asparagine pentafluorophenyl ester. The rest of the amino acids were coupled via the HATU/DIEA/DMF in situ activation.

We explored two polymer supports based on macroporous polystyrene: PS200 (Amersham Biosciences) and ArgoPore™ (Aldrich). The supports were functionalized by sarcosine followed by an aminohexyl succinate linker.[3] The resins were subjected to automated peptide assembly, except for the asparagine residue which was coupled manually. In the case of peptide **2**, after assembly the 2-chlorotrityl group was removed by mild acid treatment, and the resin was capped using isobutyric anhydride to protect the phenolic group of tyrosine. All the peptide assemblies were followed by Fmoc-Hse(Trt)-OH coupling and an optional fluorescein label could be introduced.

Then, peptide-loaded resins were subjected to automated phosphoramidite oligonucleotide synthesis. Conjugates were cleaved from the resin and deprotected by conc. ammonia at 55°C overnight. The resulting products were isolated in good yield and analyzed by reversed-phase HPLC, and their respective molecular masses confirmed by MALDI-TOF mass spectrometry.

In conclusion, we have presented a new approach towards the total stepwise solid-phase synthesis of peptide-oligonucleotide conjugates based on our homoserine linker.[3] This approach is an effective and expeditious way to obtain cell-penetrating peptide-oligonucleotide conjugates necessary for antisense inhibition and cell delivery studies.

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