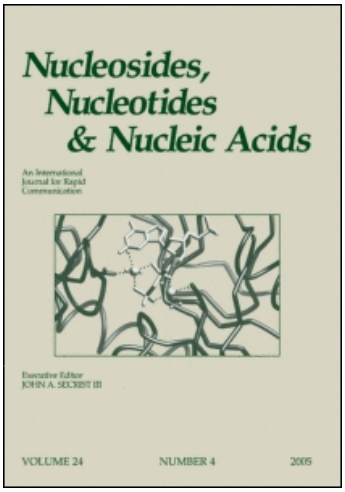

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A Convenient Solid-Phase Method for the Synthesis of Novel Oligonucleotide-Folate Conjugates

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A CONVENIENT SOLID-PHASE METHOD FOR THE SYNTHESIS OF NOVEL OLIGONUCLEOTIDE-FOLATE CONJUGATES

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□ *We describe the preparation of two batches of a polymer support for the incorporation of folic acid into oligonucleotides. The method permits the regioselective attachment of a target nucleic acid sequence through its 3′-end to either the α- or γ-carboxyl group of L-glutamic acid, respectively. The supports have been tested in solid-phase synthesis of oligonucleotide-folate conjugates for cell delivery studies.*

Keywords Oligonucleotide; folic acid; conjugate; solid support

INTRODUCTION

Covalent conjugation of nucleic acids to various functional molecules is being studied extensively in order to improve cell-specific targeting and cellular delivery of oligonucleotides for sequence-specific modulation of gene expression. A promising approach for the enhancement of cell uptake of oligonucleotides is to exploit the active transport mechanism of receptor-mediated endocytosis.[1] It has been found[2] that the covalent attachment of folic acid to an oligonucleotide produces a conjugate capable of internalization into folate receptor-bearing cells in a similar fashion to that of the free folic acid. Therefore, it would be advantageous to design novel oligonucleotide-folate conjugates and

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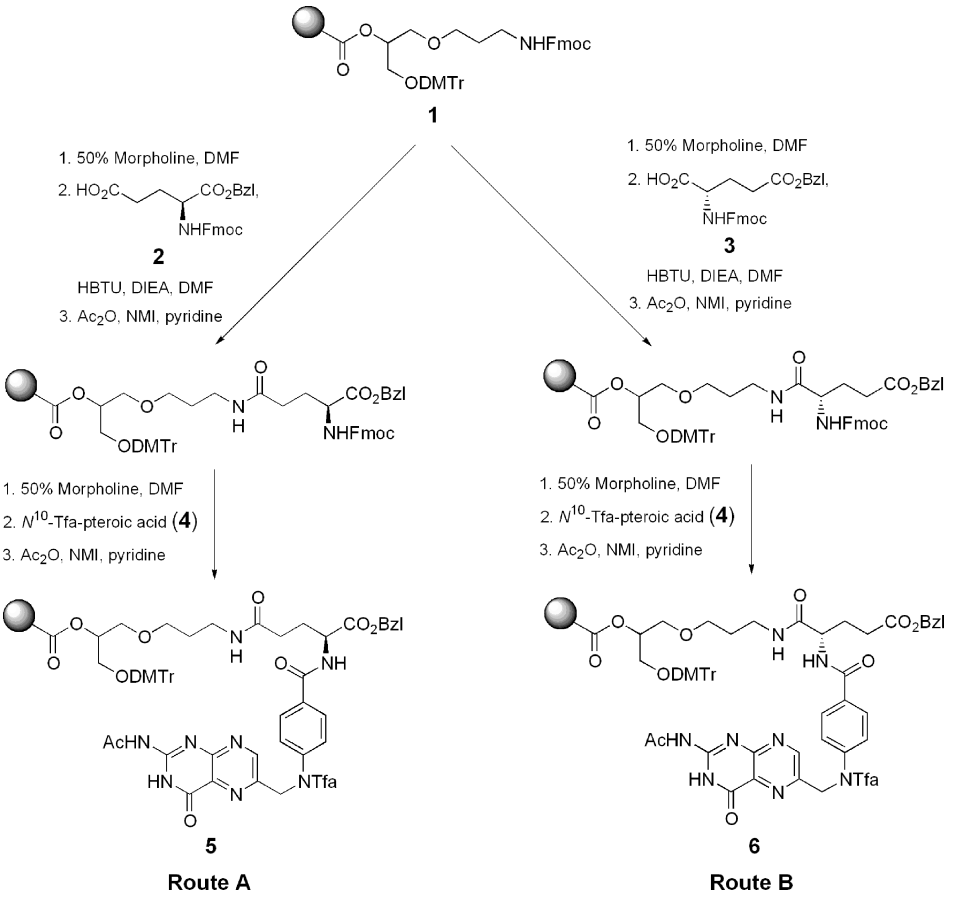

SCHEME 1 Synthesis of the folate-linked solid supports.
(**Abbreviations:** Bzl = benzyl; DIEA = *N,N*-diisopropylethylamine; DMTr = 4,4′-dimethoxytrityl; Fmoc = 9-fluorenylmethoxycarbonyl; HBTU = benzotriazol-1-yloxy-*N,N;N′,N′*-tetramethyluronium hexafluorophosphate; NMI = *N*-methylimidazole; Tfa = trifluoroacetyl.)

develop new chemical methods for their assembly. Here we report our preliminary results on the synthesis of the 3′-folate conjugates of oligodeoxyribonucleotides.

RESULTS AND DISCUSSION

Modification of the 3′-end of an oligonucleotide is important for the design of novel diagnostic probes and antisense oligonucleotides. One of the useful modifications of the 3′-end is the introduction of a 3′-bifunctional linker, e.g. the one which can be used for the attachment of a variety of pendant groups like fluorescent dyes, biotin or other molecules, that is, peptides, to oligonucleotides via solid-phase synthesis.[3,4] Here, we wish to

report the modification and extension of the method[4] toward solid-phase synthesis of oligonucleotide conjugates with folic acid.

In our first experiments, previously functionalized 500 Å long-chain alkylamine controlled pore glass (LCAA-CPG) support (**1**) was adopted for a stepwise assembly of folic acid (Scheme 1). Folic acid was assembled by an HBTU-mediated coupling of either α-benzyl ester (**2**) (Scheme 1, route A) or γ-benzyl ester (**3**) (Scheme 1, route B) of *N*α-Fmoc-L-glutamic acid followed by the Fmoc deprotection by treatment with morpholine and the second coupling with N^{10}-trifluoroacetylated pteroic acid (**4**). Both couplings were followed by the acetic anhydride/*N*-methylimidazole/pyridine capping. Synthesis efficiency was monitored by spectrophotometric Fmoc removal test at 301 nm, and double couplings were used when the yield dropped below 95%. The average stepwise yield was >96%. This approach permits the regioselective coupling of folic acid to oligonucleotides through either the α- or γ-carboxyl group of L-glutamic acid. However, it has been demonstrated previously that only the conjugates attached via the γ-carboxyl of folic acid retain the ability to bind to the cell surface folate receptors with the same affinity as the free folic acid.[5] Therefore, the route B (Scheme 1) was used as a model to optimise the reaction conditions. The conjugates produced by the route B may be used as controls to compare the properties of the α- and γ-linked folate oligonucleotides.

The folate-loaded supports (**5**) and (**6**) were subjected to the standard oligonucleotide assembly by the cyanoethyl phosphoramidite method on an ABI 394 DNA Synthesizer with 2′-deoxyribonucleoside 3′-phosphoramidites, in accordance with the manufacturer′s protocols. The average coupling yields were >97%. The conjugates were cleaved from the supports and treated with 1M NaOH first to prevent the ammonolysis of the benzyl ester followed by concentrated aqueous ammonia at 55°C overnight. The products were isolated in good to moderate yield and analysed by reversed-phase HPLC in ion-pair mode and MALDI-TOF mass spectroscopy. This part of the work is in progress and will be reported in due course.

CONCLUSIONS

We have described the preparation of two differently functionalized CPG supports for the regioselective incorporation of folic acid into an oligonucleotide sequence at the 3′-end through the α- or γ-carboxyl group of L-glutamic acid. The supports can be applied for solid-phase synthesis of oligonucleotide-folate conjugates that may possess improved cell uptake properties.

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