Pergamon

Tetrahedron Letters, Vol. 38, No. 3, pp. 417-420, 1997
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0040-4039/97 $17.00 + 0.00

PII: S0040-4039(96)02314-3

Nucleic Acid Analog Peptide (NAAP). Solid Phase Synthesis of a DNA Analog Peptide

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Abstract: Solid phase synthesis of a nucleic acid analog peptide (NAAP), as a substitute for antisense or triple-helix forming oligonucleotide, is described. The N-protected amino acid monomer was prepared starting from 3'-azido-3'-deoxythymidine (AZT) in 20 % overall yield. Condensation of the amino acid monomer was performed using Boc-chemistry on MBHA resin to afford a decapeptide containing 10 thymine bases in 21 % overall yield.
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Development of artificial regulatory molecules for a specific gene expression has been of special interest from the medicinal and biological points of view.[1] In particular, the so-called genetic medicines such as antisense or triple-helix forming oligonucleotides, ribozymes, and decoy RNAs are promising candidates for harmless therapeutic agents or research tools in molecular biology.[2] A number of chemical modifications have been made on such synthetic DNAs and RNAs in order to overcome some inherent drawbacks, including degradability by cellular nucleases, impermeability into a cell membrane, and low hybridization affinity caused by electrostatic repulsion between phosphate backbones.[3] Among the modifications, introduction of a peptide backbone into the modified nucleic acid analogs seems to be a rational and attractive challenge because the electrically neutral character of the peptide backbone may improve the nuclease resistance, membrane permeability and hybridization affinity of the analogs. Moreover, the peptide analogs can be easily conjugated with various functional molecules such as labeling agents, intercalators, catalytic reagents and certain peptides or proteins with specific functions. Recently, some reports have been made on the introduction of a peptide backbone into oligonucleotides and their hybridization properties with ssDNA and ssRNA.[4-6] In these studies, dimeric derivatives **2-5** with an amide function as shown in Figure 1 were incorporated into oligonucleotides in which the rest of the linkages consisted of normal phosphate diester bonds, and these modified oligonucleotides showed comparable or somewhat lower hybridization affinities with complementary ssRNAs compared to the wild type oligo DNAs. Such partial modifications of

the backbone, however, do not always show the effects realized by the entire replacement of the phosphate linkages in the oligonucleotides as demonstrated by S. Tam and his coworkers.[7] The present study describes the synthesis of a novel DNA analog **1** bearing all the peptide linkages on solid phase using Boc-chemistry.

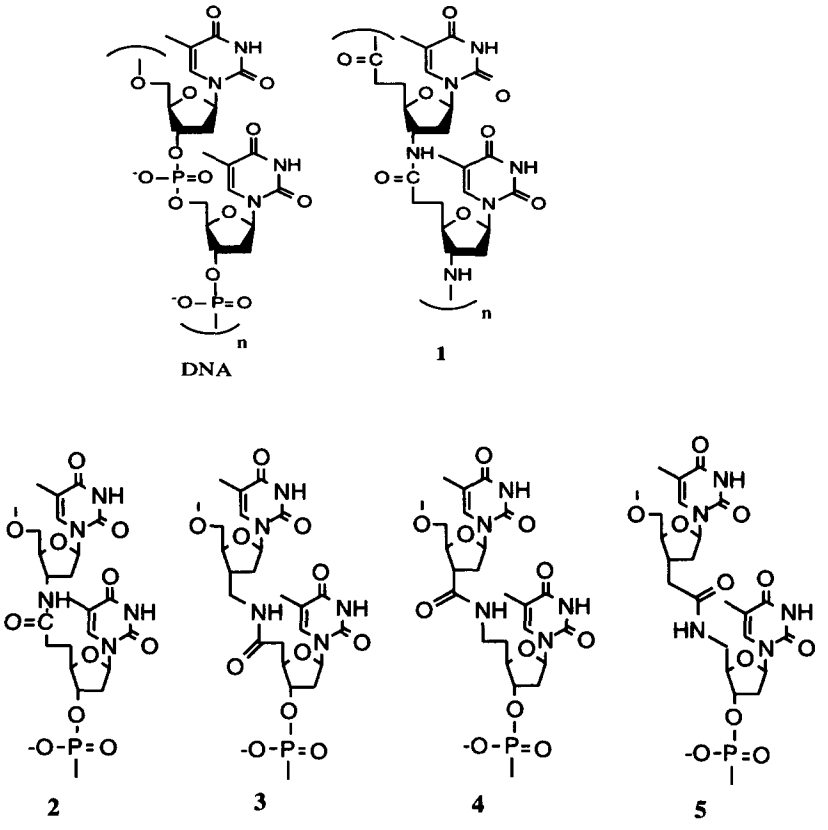
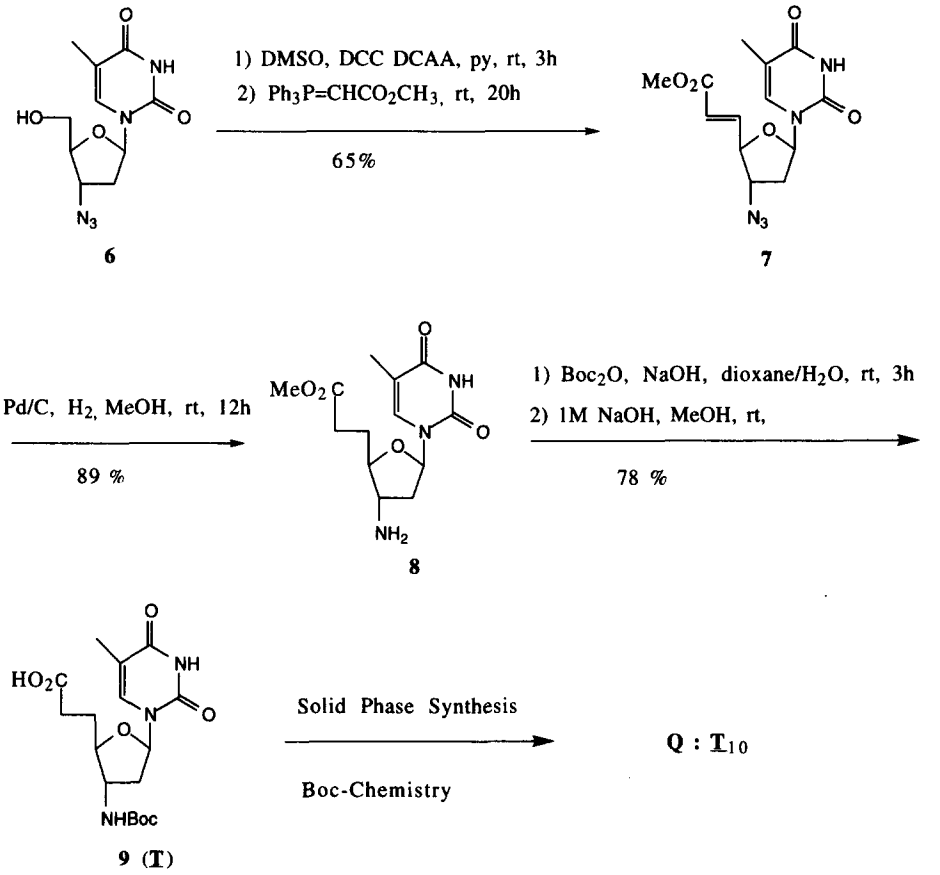


Figure 1. DNA Analogs with a Peptide Backbone

The NAAP **1** bearing the 3-amino-β-D-riboheptanofuranuronate moiety was prepared as shown in Scheme 1. Preparation of 3'-azido-3'-deoxythymidine (**6**, AZT) was carried out as described in the literature.[8] Oxidation of **6** with dimethyl sulfoxide in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and dichloroacetic acid (DCAA) gave the corresponding aldehyde, which was subsequently treated with

methoxycarboxylmethylene triphenylphosphorane in one pot.[9] The product, methyl 3-azido-1,2,3,5,6-pentadeoxy-1-(thymin-1-yl)-β-D-ribohept-5-eno-furanuronate (**7**), was isolated in 65 % overall yield. Catalytic hydrogenation of **7** on palladium charcoal afforded amino ester derivative **8** in 89 % yield. After protection of the amino function with the Boc group, hydrolysis of the resulting ester with 1M sodium hydroxide at room temperature gave N-Boc amino acid **9** in 78 % yield. Thus, the synthesis of the N-protected amino acid monomer was achieved in 20 % overall yield. Solid phase synthesis using Boc-chemistry on MBHA resin[10] gave decapeptide **Q** containing ten thymine bases in 21 % overall yield (ca. 84 % on average for each coupling) as a pure form after reversed phase HPLC purification. (Megapak SIL C18-10, 10 mm, 10 x 250 mm, 20-50 % CH_3CN linear gradient in 0.1 M aqueous ammonia acetate, pH 4.5) The obtained peptide **Q** was confirmed by FAB mass spectrometry; mass for **Q** $C_{120}H_{153}N_{31}O_{40}$ m/z calcd 2669.6, found 2670.6 [$(M+H)^+$].



Scheme 1. Synthesis of a NAAP **1** Decamer (**Q**)

The obtained DNA analog decapeptide **Q** bearing 10 thymine bases has the potential to be an antisense and antigene molecule. In addition to the obvious resistance against cellular nucleases, the analog can be expected to have cell membrane permeability because of its electrostatically neutral backbone. In our preliminary studies on the hybridization properties with RNA, **Q** and the complementary oligoribonucleotide rA_{10} showed no detectable hypochromic effect on UV spectra at 260 nm (50 mM Tris, pH 7.0, 20 mM $MgCl_2$, 100m M NaCl). More detailed results on the double or triple strand formation by **Q** with ssDNA and ssRNA as well as dsDNA will be published elsewhere. Further studies on the biological properties such as stability in a serum, and cell membrane permeability as well as on the synthesis of cytidine analog are also now in progress in our laboratory.

ACKNOWLEDGMENTS

The authors express their gratitude for the financial support from Chugai Pharmaceuticals Award in Synthetic Organic Chemistry, Japan and also from Japan Private School Promotion Foundation.

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(*Received in Japan* 15 *October* 1996; *revised* 19 *November* 1996; *accepted* 22 *November* 1996)