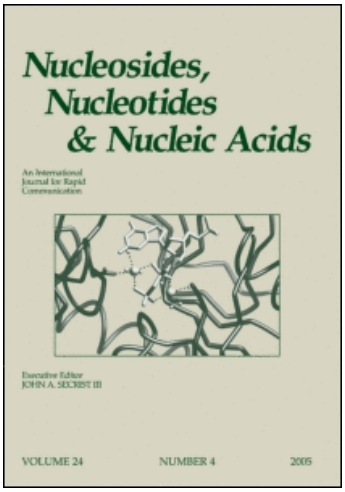

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Synthesis of Novel Polydiamidopropanoate Dendrimer PNA-Peptide Chimeras for Non-Invasive Magnetic Resonance Imaging of Cancer

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SYNTHESIS OF NOVEL POLYDIAMIDOPROPANOATE DENDRIMER PNA-PEPTIDE CHIMERAS FOR NON-INVASIVE MAGNETIC RESONANCE IMAGING OF CANCER

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▫ *A variety of dendrimers can be conjugated to oligonucleotides to increase the number of contrast paramagnetic atoms (e.g., gadolinium or dysprosium) per probe. Thus, it was of interest to test a route for assembly of chelating dendrimer branches directly on the N-termini of peptide nucleic acid (PNA)-peptide chimeras by continuous solid-phase coupling on polymer supports. Dendrimer-PNA-peptides complementary to 12 nt of mutant KRAS mRNA have been prepared with a C-terminal insulin-like growth factor 1 (IGF1) analog d(Cys-Ser-Lys-Cys) and N-terminal polydiamidopropanoate (PDAP) dendrimers with different numbers of diaminopropanoate residues. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelating moieties were then coupled to PDAP dendrimer-PNA-peptide chimeras before cleavage from the polymer supports. The DOTA-PDAP-PNA-peptide probes with 1, 2, 4, 8, or 16 amino (or DOTA) moieties were cleaved, purified by RP-HPLC, and characterized by MALDI-TOF mass spectroscopy.*

INTRODUCTION

One can envision non-invasive magnetic resonance imaging (MRI) of mRNAs in cells in tumors by hybridization of complementary oligonucleotides conjugated to diamagnetic metals as contrast agents. However, there are usually too few oncogene mRNAs per cell to allow detection by MRI at one metal ion per probe.[1,2]

To increase the sensitivity of MRI, the chemically synthesized polydiamidoamine (PAMAM) dendrimers, conjugated to metal cation chelating reagents like

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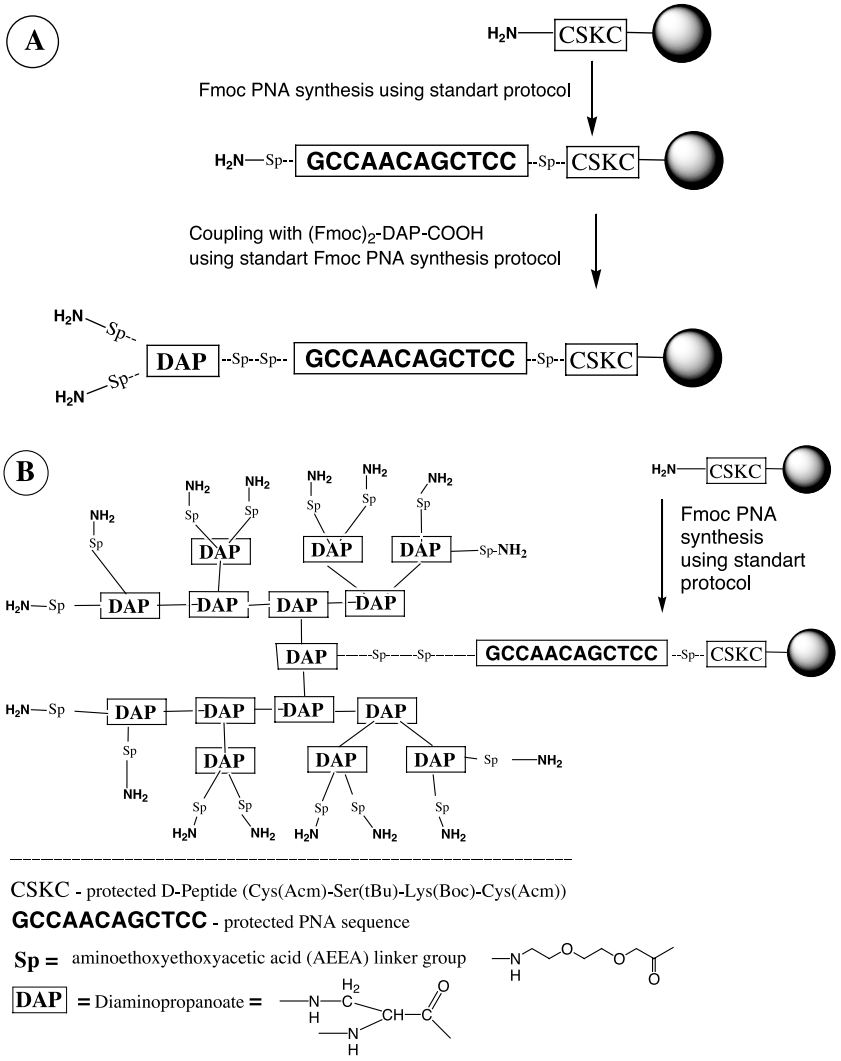
diethylenetriaminepentaacetic acid (DTPA) or DOTA, have been attached to various peptides or antibodies in aqueous buffer solutions.[3,4] In these studies, pH 8–9 was necessary to deprotonate reactive amino groups of the peptides or antibody. The same chemical approach cannot be used to prepare PAMAM dendrimer PNA-peptide chimeras because of the low solubility of PNA or PNA-peptides in water solution at pH 8–9. In the current study, we assembled dendrimer branches directly on polymer supports continuously by coupling Fmoc-protected diaminopropanoic acid (DAP) monomers and Fmoc-protected



SCHEME 1 Solid-phase synthesis of PDAP dendrimer PNA-peptide chimeras with 2 (**A**) and 16 (**B**) amino groups.

aminoethoxyethoxyacetic acid (AEEA) monomers after solid phase synthesis of PNA-peptide chimeras on polymer supports.

The polydiamidopropanoate (PDAP) dendrimer PNA-peptide chimeras, $(H_2N\text{-}AEEA)_n\text{-}PDAP^m\text{-}AEEA_2\text{-}$**GCCAACAGCTCC**-AEEA-D(Cys-Ser-Lys-Cys)-$C(O)NH_2$, with different generations of PDAP dendrimers (m = 1, 2, 3, or 4) and different numbers of free amino groups on the ends of PDAP dendrimer (n = 2, 4, 8, or 16) were synthesized using solid-phase synthesis on an Expedite 8909 automatic DNA synthesizer (Applied Biosystems, Foster City, CA), as illustrated in Scheme 1. The macrocyclic chelator, DOTA, was conjugated to the each free amino groups of PDAP dendrimer. A protected DOTA analogue, 1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid *tert*-butyl ester)-10-acetic acid (DOTA-3tBu) (Macrocyclics, Richardson, TX), was activated by O-(7-azabenzo-triazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and coupled to the designated position of the PDAP dendrimer on PNA-peptide. Activation was accomplished by mixing monomer, HATU, and base solution containing diisopropylamine (DIEA) and lutidine in DMF.

The subsequent trifluoroacetic acid treatment not only cleaved the PDAP-PNA-peptide or DOTA-PDAP-PNA-peptide off the resin but also removed all protecting groups from the PNA and peptide as well as DOTA-3tBu. The PDAP-PNA-peptide chimeras was purified by reversed-phase high-pressure liquid chromatography (HPLC) (Figure 1) and characterized by mass spectrum, MALDI-MS (Figure 2); (M + H) + : for $(H_2N\text{-}AEEA)_n\text{-}PDAP^m\text{-}AEEA_2\text{-}$**GCCAACAGCTCC**-AEEA-D(Cys-Ser-Lys-Cys)-$C(O)NH_2$, n = 2, m = 1, MW 4430.8 (calcd), 4428.4 (found); n = 4, m = 2, MW 4893.3 (calcd), 4890.5 (found);. n = 8, m = 3, MW 5818.2 (calcd), 5817.9 (found); n = 16, m = 4, MW 7668.0 (calcd), 7667.6 (found). For (DOTA-$C(O)N(H)\text{-}AEEA)_n\text{-}PDAP^m\text{-}AEEA\text{-}AEEA\text{-}$**GCCAACAGCTCC**-AEEA-D(Cys-Ser-Lys-Cys)-$C(O)NH_2$, n = 2, m = 1, MW 5203.2 (calcd), 5205.3 (found); for n = 4, m = 2; n = 8, m = 3, and n = 16, m = 4 MALDI mass spectra are undetectable.

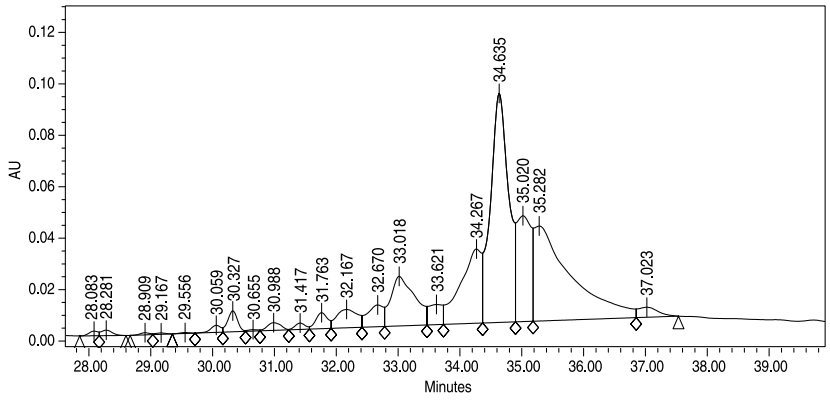


FIGURE 1 Preparative C_{18} HPLC profile of the solid-phase coupled PDAP dendrimer PNA-peptide chimeras $(H_2N\text{-}AEEA)_{16}\text{-}PDAP^4\text{-}AEEA\text{-}AEEA\text{-}PNA\text{-}AEEA\text{-}$peptide after cleavage from the support.

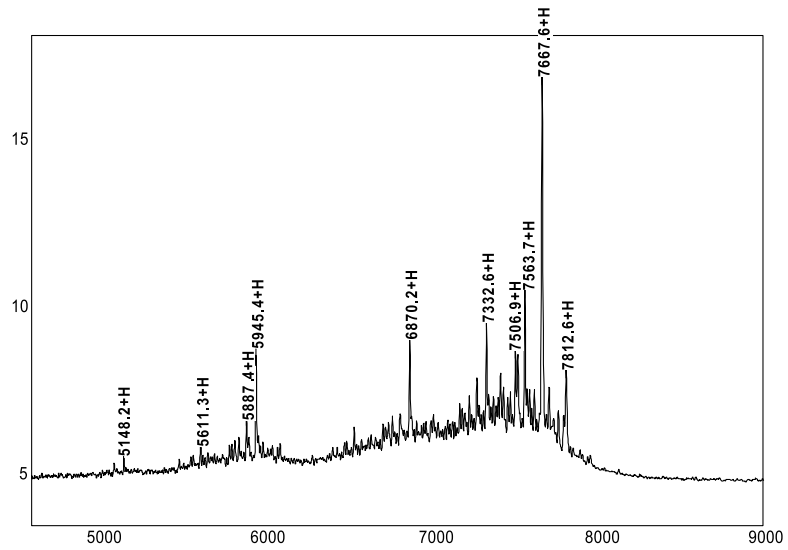


FIGURE 2 MALDI-TOF mass spectrum of the solid-phase coupled PDAP dendrimer PNA-peptide chimeras $(H_2N\text{-AEEA})_{16}$-PDAP^4-AEEA-AEEA-PNA-AEEA-peptide after cleavage from the support and HPLC purification. MW: 7668.0 (calculated), 7667.6 (found).

Thus, PNA-peptide probes with 1, 2, 4, 8, or 16 amino (or DOTA) moieties have been synthesized on polymer supports, cleaved and purified by HPLC, and characterized by MALDI-TOF mass spectroscopy. The chelation of Gd(III) with the DOTA moieties of these chimeras and MRI experiments with the new DOTA-PDAP dendrimer PNA-peptide chimeras are underway. In this work we demonstrated the possibility to assemble polyamino dendrimer PNA-peptide molecules directly on polymer supports.

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