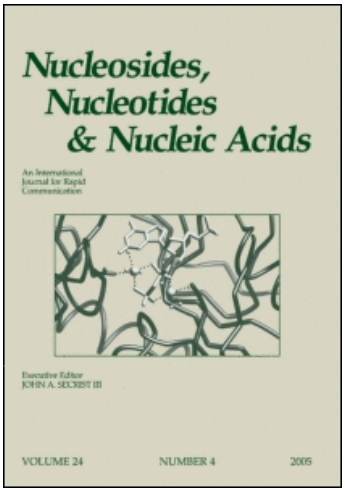

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LARGE SCALE HPLC PURIFICATION OF OLIGODEOXYRIBONUCLEOTIDES FOR STUDIES OF THE BINDING OF CC-1065 TO DNA BY NMR SPECTROSCOPY

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ABSTRACT. We have investigated four different HPLC columns and several conditions in search of the most efficient procedure for purification of milligram amounts of DNA. We are reporting the optimal conditions for a preparative purification using a Dupont Zorbax Bioseries Oligo column.

The synthesis of oligodeoxyribonucleotides is now fairly routine on commercially available DNA synthesizers using the solid-phase phosphoramidite triester coupling approach. Polyacrylamide gel electrophoresis (PAGE) and high-pressure liquid chromatography (HPLC) by ion-exchange or reverse-phase modes[2] are the most commonly used methods for the purification of oligonucleotides. However, the study of drug-DNA interactions by NMR spectroscopy and X-ray crystallography is becoming increasingly important. Obtaining the level of purity and the large quantities needed for these studies is not straightforward. With these qualifications in mind, we examined four analytical HPLC columns, Nucleogen DEAE-60[3], Vydac C_4[4], Supelco LC-Si[5,6] and Dupont Zorbax Bioseries Oligo, under several conditions in search of the most efficient procedure for purification of milligram amounts of oligonucleotides. Eight oligonucleotides, varying in length from six to thirteen bases, and identical from the 3'-terminus (5'd(GCGCGAATTCGCG) 3'), were synthesized using the solid- phase methoxy-phosphoramidite triester coupling approach[7] on a 380B ABI DNA synthesizer. The 12-bases oligomer was of interest to us because it is known to bind CC-1065, a potent antitumor antibiotic, reversibly[8].

The Vydac C_4 and Supelco LC-Si columns gave relatively good resolutions but unpredictable elution orders. The Supelco LC-Si column was successfully used earlier for the purification of non-self complementary 7- and 8-mers. Although the Nucleogen DEAE column gave good separation and relatively consistent retention times, the best results, in terms of both resolution and predictability, were obtained on the Zorbax Oligo column. Oligos up to thirteen bases in length could be baseline separated. The outstanding performance of the analytical Zorbax column led us to investigate a preparative scale Zorbax column. Excellent results were obtained utilizing a sodium triphosphate-sodium chloride-acetonitrile buffer system. The column was successfully used to purify up to 300 O.D. units of material per injection and is therefore capable of purifying the product of a 10umole synthesis in two injections to generate enough material for NMR studies. The oligonucleotides were successfully desalted on a Sephadex G-25 column using water as elution buffer and then passed through a Na^+ resin. ^{1}HNMR spectra of the self-complementary dodecanucleotide duplex (5'd(CGCGAATTCGCG)$_2$ 3') and a non-self-complementary decamer duplex (5'd(CGCGATTA*GC-GCTAATCGCG) 3') were run. The non-covalent interaction of the dodecamer and the covalent interaction of the decamer (A* is the site of covalent interaction) with CC-1065 were confirmed by a battery of 2-D experiments including COSY, NOESY and HOHAHA[9].

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