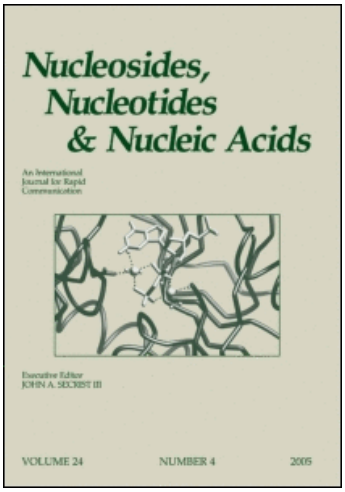

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HPLC and Synthesis Strategy in Nucleoside and (Oligo)Nucleotide Chemistry

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HPLC AND SYNTHESIS STRATEGY IN NUCLEOSIDE AND (OLIGO)NUCLEOTIDE CHEMISTRY

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Abstract. A judicious use of HPLC allows to simplify the synthetic approach of an (oligo)nucleotide. As an example, is reported a preparation of an antiviral (3'-5')dinucleotide with simultaneous isolation of its (3'-3') isomer.

The common way to synthesize a definite nucleoside or (oligo)nucleotide consists in a sequence of selective reactions with purification of each intermediate by conventional processes.

Extensive use of H.P.L.C. led us to a straightforward approach: starting from more simple materials and/or using less selective reactions, the separation by preparative HPLC of the final mixture could allow to obtain the required product with improvement in time, cost and yield. Simultaneously by-product could be isolated and investigated.

As an example, we report a convenient preparation of arabinofuranosyladenylyl (3'-5') arabinofuranosyladenine (Compound A which proved to have an antiviral activity) with simultaneous isolation of its 3'-3' isomer (Compound B).

In a previous work[1], compound A have been unambiguously synthesized by the phosphotriester method in liquid phase. Starting from 9-β-D-arabinofuranosyl adenine (ara-A), the yield was 13% after ten steps.

In the present approach, the number of steps was reduced to six, with purification of only the first three interme-

diates and separation of the final mixture by isocratic reversed-phase HPLC.

SYNTHESIS. N^2,2'-O-di(monomethoxytrityl) and N^2,2',5'-tri-(monomethoxytrityl) derivatives of ara-A were prepared as previously described[1] . The phosphorylation product of the second one by a H-phosphonate method[1] was condensed directly with the first one. The resulting mixture of phosphonate derivatives was oxydized and acid-treated. After extraction, the crude mixture of A and B was HPLC-analyzed and separated

ANALYTICAL HPLC. Isocratic reversed-phase HPLC was performed on a WATERS-MILLIPORE apparatus equipped with an UV-diode-array detector. Stainless column (150 mm * 3.9 mm ID) was fitted with Nucleosil C18, 3 µm particle size (SFCC). Eluent was 0.1M ammonium acetate,acetonitrile 8% (flow rate 0.8 ml/mn,30°C).Analysis was complete in 15 minutes,showing the presence of A,B and ara-A (same retention times and UV-spectra than authentic samples).

PREPARATIVE HPLC. Nucleosil C18, 10µm particle size and the same eluent were used in a stainless column (150 mm * 19 mm ID,flow rate 9 ml/mn,20°C). The WATERS-MILLIPORE apparatus consisted of modules 510EF,720,730,U6K,681 and R401. Chromatogram was comparable to the analytical one and the required fractions were collected,desalted and analyzed.

A CONVENIENT DESALTING METHOD.Pooled fractions of the purified product were partially reduced for eliminate most of the acetonitrile,then the resulting solution (10-50 ml) was directly injected by means of a pump in the same preparative C18 column,previously equilibrated with water. The column was washed with pure water until complete elution of the salts,as monitored by the refractometer. At this time,a linear gradient of 0-20% acetonitrile in 5 minutes was programmed. The required fraction (UV-monitored) was collected and evaporated to dryness. Yield and time are better than those obtained by evaporation of salts or by injection of a concentrated solution in the HPLC column by means of an injector. This method may be useful to eliminate non-volatile salts.

CONCLUSION. Improved time and yield (A: 38%, B: 2%, overall 40% from ara-A). Simultaneous isolation of by-product.

(1) F. Puech, G. Gosselin, J. Balzarini, E. De Clercq, J.L. Imbach, J. Med. Chem., 1988, **31**, 1897.