Tetrahedron Letters, Vol.31, No.26, pp 3673-3676, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

Separation of 5'-Dimethoxytrityl-2'-Deoxynucleoside-3'-O-Methylphosphonamidite Diastereomers by Normal Phase Chromatography

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Abstract: Rp and Sp diastereomers of the 5'-dimethoxytrityl-2'-deoxynucleoside-3'-O-methylphosphonamidites of N^6-Benzoyl-adenine, N^2-isobutyryl-guanine, N^4-benzoyl-cytosine, and thymine were separated by liquid chromatography on a normal phase silica column eluted with 1.0% EtOH in $CHCl_3$.

The efficacy of oligodeoxynucleoside methylphosphonates as antisense inhibitors of oncogene or viral gene expression [1] is limited by the racemic heterogeneity of the internucleotide linkages [2,3]. Stereospecific synthesis of all-R oligomers may significantly increase the antisense efficacy of oligodeoxynucleoside methyl-phosphonates [4-7]. However, in order develop a stereospecific coupling reaction scheme, via a trivalent phosphonamidite pathway, it is essential to first separate the commercially available 5'-dimethoxytrityl-2'-deoxynucleoside-3'-O-methyl-phosphonamidite diastereomers [8]. While pursuing synthetic work in this area we have developed a new nucleophile catalyzed phosphonamidite coupling reaction [9] which referred to an unpublished method for separating the diastereomers. Here, we report the successful separation and isolation of the diastereomers, dependent upon pretreatment of the silica gel column with Et_3N.

Methods

Analytical liquid chromatography was performed using an Alltech 4.6 x 250 mm Econosphere 5 micron silica column initially equilibrated with 1% Et_3N (99%, Aldrich #13,206-3) in $CHCl_3$ (HPLC grade, Fisher # FI C-606-4, containing 1% EtOH) for 10 min. The column was then washed with 2.5% EtOH in $CHCl_3$, prepared by adding 1.5% (v/v)

absolute EtOH USP (Aaper Alcohol and Chemical Co.) to HPLC grade $CHCl_3$ containing 1% EtOH, for 10-20 minutes at a flow rate of 1.0 ml/min. Finally, the column was equilibrated with HPLC grade $CHCl_3$ containing 1% EtOH. The solvents were used without additional pretreatment. Two milligrams of either 5'-dimethoxytrityl-N^6-Benzoyl-2'-deoxyadenosine-3'-(N,N-diisopropylamino) methylphosphonamidite (ABN, Inc.) (2.5 μmoles), **1**, 5'-dimethoxytrityl-N^2-isobutyryl-2'-deoxyguanosine-3'-(N,N-diisopropylamino) methylphosphonamidite (ABN, Inc.) (2.5 μmoles), **2**, 5'-dimethoxytrityl-N^4-benzoyl-2'-deoxycytidine-3'-(N,N-diisopropylamino) methylphosphonamidite (ABN, Inc.) (2.5 μmoles), **3**, or 5'-dimethoxytrityl-2'-deoxythymidine-3'-(N,N-diisopropylamino) methylphosphonamidite (Genta, Inc.) (2.9 μmoles), **4**, were dissolved in 150 μl each of $CHCl_3$. For each analytical run, 5-10 μl of each solution was applied to the column, which was then eluted with 1% EtOH in $CHCl_3$ at 1.0 ml/min. (Fig. 1).

Preparative separation of the phosphonamidite diastereomers was performed by applying 40-50 mg samples to an Alltech 2.25 x 25 cm RSil 10 micron silica column. A single preparative run was sufficient for virtually complete separation of the diastereomers. Tailing of **2** required a second run of each peak. Recovery of the diastereomers appeared to be quantitative. The column was pretreated as described above, except that the flow rate was 16 ml/min. The products of the separation were

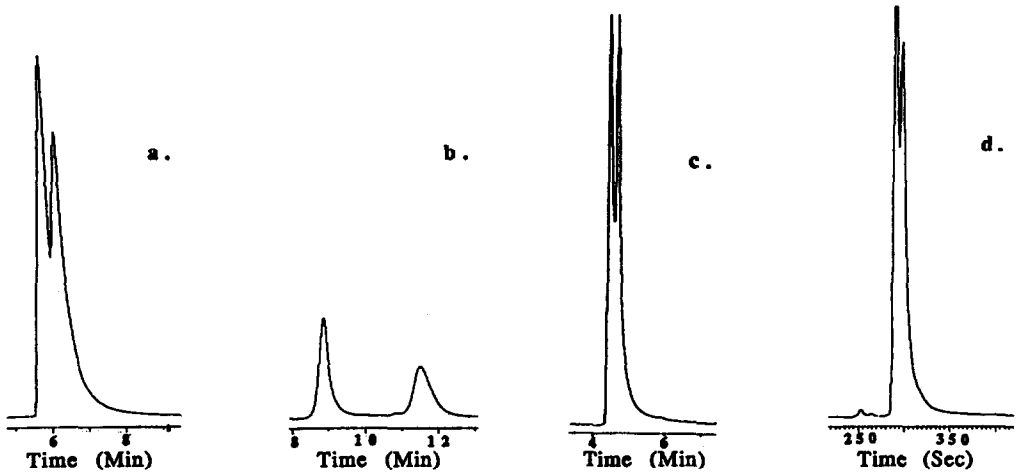

Figure 1: Analytical liquid chromatographic separations of the diastereomers of **1** (a), **2** (b), **3** (c), and **4** (d).

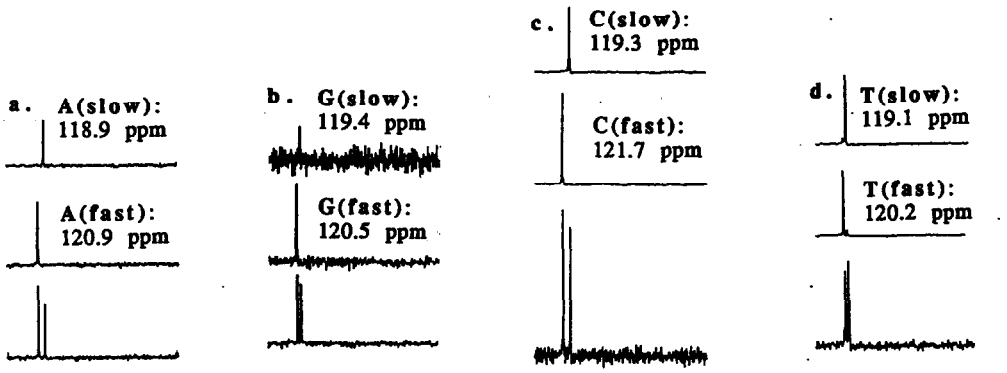

Figure 2: ^{31}P NMR of the diastereomers before and after preparative separation. All samples were dissolved in CD_3CN. Listed with the separated diastereomers are the chemical shifts of **1** (a), **2** (b), **3** (c), and **4** (d). The chemical shifts are relative to H_3PO_4.

analyzed by ^{31}P NMR in CD_3CN, using a Jeol FX-90Q spectrometer (Fig. 2). The chemical shifts were measured relative to an external standard of dimethyl methylphosphonate in CD_3CN (32.4 ppm from H_3PO_4). ^{31}P NMR indicated that the diastereomers were completely separated for all nucleosides, except for **4** which showed <5% impurity. It was observed that there was a correlation between the retention times and the ^{31}P chemical shifts. For all nucleosides studied, the faster eluting diastereomer gave a larger ^{31}P chemical shift when compared to the slower eluting diastereomer (Fig. 2).

In our early attempts to separate the 5'-dimethoxytrityl-2'-deoxynucleoside-3'-O-methylphosphonamidite diastereomers, it was observed that the diastereomers underwent hydrolysis and oxidation. Upon pretreatment of the silica with 1% Et_3N we observed better than 95% separation with virtually no hydrolysis or oxidation for all phosphonamidites used. The separation of the Rp and Sp diastereomers of the phosphonamidites is the initial necessary step in developing a stereospecific pathway

for oligodeoxynucleoside methylphosphonate synthesis via the trivalent phosphonamidite route.

Acknowledgments: This work was supported by grants to E.W. from the National Cancer Institute, the Florida High Technology and Industry Council, and Genta, Inc.

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(Received in USA 21 February 1990)