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# SOLUTION SYNTHESIS OF PROTECTED DI-2'-DEOXYNUCLEOSIDE PHOSPHOTRIESTERS VIA THE PHOSPHORAMIDITE APPROACH

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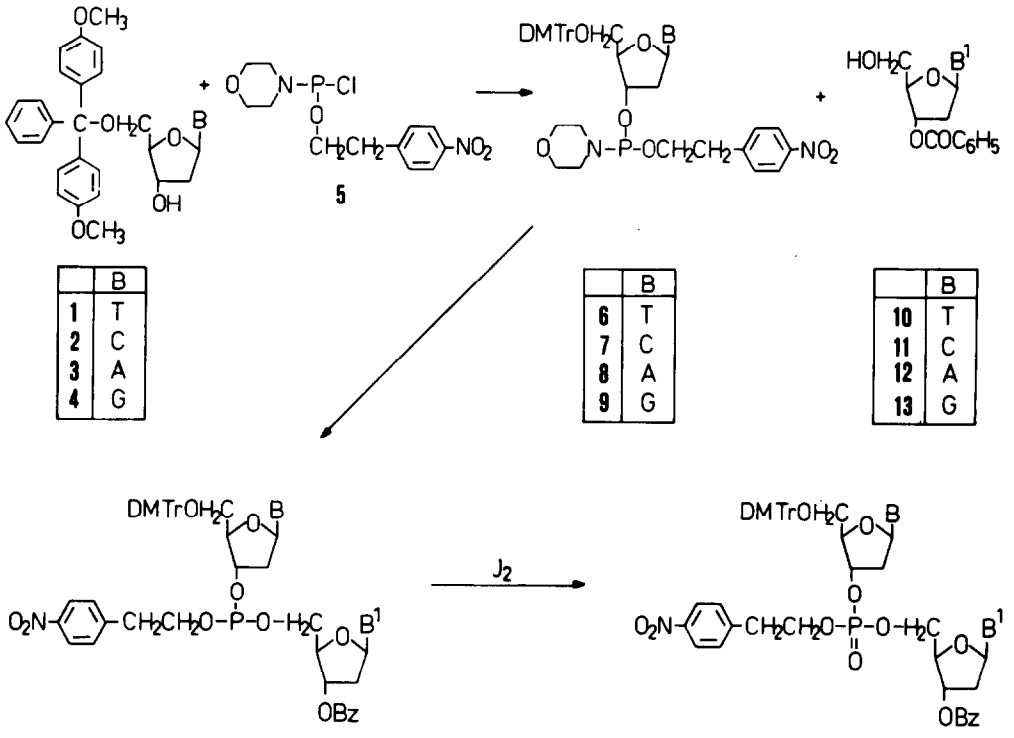
The synthesis of fully protected di-2'-deoxyribonucleoside-3'-[2-(4-nitrophenyl)ethyl] phosphates (16-31) via the phosphoramidite approach in solution is described and the products characterized by spectrophotometrical and chromatographical means.

The newest and most successful approach in polymer supported deoxyoligonucleotide synthesis has recently been introduced by Caruthers [1,2] using deoxynucleoside phosphoramidites as a new class of highly reactive monomeric building blocks. These key intermediates are derived from appropriately protected deoxynucleosides and carry mainly the methoxy as well as the cyanoethyl group [3] as ester functions, whereas the amidite grouping variesmore broadly including the dimethylamino [1,4], diethylamino [4], diisopropylamino [2,4], pyrrolidino [2], 2,2,6,6-tetramethylpiperidino [2] and morpholino [2,3,5] group.

The striking good features of the p-nitrophenylethyl (NPE) group for phosphate protection [6-8] in oligonucleotide synthesis encouraged us to investigate systematically the Caruthers method with the modified deoxynucleoside p-nitrophenylethyl phosphoromorpholidites (6-9) in solution synthesis to form with the 3'-O-benzoyl-2'-deoxyribonucleosides (10-13) the corresponding di-2'-deoxynucleoside phosphotriesters (16-31) in preparative scale.

The monofunctional phosphitylating agent chloro-morpholino-2-(4-nitrophenyl) ethoxyphosphine (5) was prepared from an equimolar mixture of dichloro-2-(4-nitrophenyl)ethoxyphosphine [9] and N-trimethylsilyl-morpholino by stirring



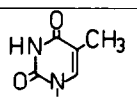
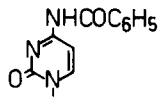
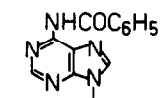
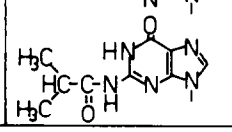
| | B |
|---|---|
| 1 | T |
| 2 | C |
| 3 | A |
| 4 | G |

| | B |
|---|---|
| 6 | T |
| 7 | C |
| 8 | A |
| 9 | G |

| | B |
|---|---|
| 10 | T |
| 11 | C |
| 12 | A |
| 13 | G |



| | B | $B^1$ |
|---|---|---|
| 14 | T | T |
| 15 | $C^{Bz}$ | $C^{Bz}$ |

| B | |
|---|---|
| T |  |
| $C^{Bz}$ |  |
| $A^{Bz}$ |  |
| $G^{iBu}$ |  |

| | B | $B^1$ |
|---|---|---|
| 16 | T | T |
| 17 | T | $C^{Bz}$ |
| 18 | T | $A^{Bz}$ |
| 19 | T | $G^{iBu}$ |
| 20 | $C^{Bz}$ | T |
| 21 | $C^{Bz}$ | $C^{Bz}$ |
| 22 | $C^{Bz}$ | $A^{Bz}$ |
| 23 | $C^{Bz}$ | $G^{iBu}$ |
| 24 | $A^{Bz}$ | T |
| 25 | $A^{Bz}$ | $C^{Bz}$ |
| 26 | $A^{Bz}$ | $A^{Bz}$ |
| 27 | $A^{Bz}$ | $G^{iBu}$ |
| 28 | $G^{iBu}$ | T |
| 29 | $G^{iBu}$ | $C^{Bz}$ |
| 30 | $G^{iBu}$ | $A^{Bz}$ |
| 31 | $G^{iBu}$ | $G^{iBu}$ |

Physical Data of Di-2'-deoxyribonucleoside Phosphotriesters

| Compound | | | Yield | UV-Absorption Spectra in MeOH | | HPL-Chromatography ($R_t$ in sec.) | | |
|---|---|---|---|---|---|---|---|---|
| | B | $B^1$ | % | $\lambda_{max}$ (nm) | lg $\varepsilon$ | $CH_2Cl_2$/MeOH (97/3) ::) | $CH_2Cl_2$/MeOH (80/20) ::) | MeOH/$H_2O$ (85/15) #) |
| 16 | T | T | 82 | 232 266 | 4.48 4.42 | 197/221 | | 140 |
| 17 | T | $C^{Bz}$ | 79 | 233 262 [304] | 4.66 4.61 [4.06] | 161/179 | | 183 |
| 18 | T | $A^{Bz}$ | 75 | 232 276 | 4.70 4.55 | 219/267 | | 202 |
| 19 | T | $G^{iBu}$ | 81 | 235 261 [274] | 4.68 4.54 [4.49] | | 399/605 | 167 |
| 20 | $C^{Bz}$ | T | 80 | 234 261 [304] | 4.68 4.63 [4.10] | 144/158 | | 172 |
| 21 | $C^{Bz}$ | $C^{Bz}$ | 79 | 235 260 300 | 4.75 4.75 4.38 | 137/156 | | 379 |
| 22 | $C^{Bz}$ | $A^{Bz}$ | 82 | 233 264 | 4.76 4.63 | 194/246 | | 266 |
| 23 | $C^{Bz}$ | $G^{iBu}$ | 84 | 237 261 | 4.69 4.68 | | 307/377 | 235 |
| 24 | $A^{Bz}$ | T | 79 | 233 274 | 4.68 4.56 | 197/205 | | 167 |
| 25 | $A^{Bz}$ | $C^{Bz}$ | 81 | 233 263 | 4.77 4.66 | 139/147 | | 252 |
| 26 | $A^{Bz}$ | $A^{Bz}$ | 75 | 233 278 | 4.73 4.65 | 185/204 | | 298 |
| 27 | $A^{Bz}$ | $G^{iBu}$ | 76 | 234 261 276 | 4.70 4.58 4.61 | | 297 | 226 |
| 28 | $G^{iBu}$ | T | 71 | 235 261 [274] | 4.60 4.55 [4.48] | | 417/545 | 157 |
| 29 | $G^{iBu}$ | $C^{Bz}$ | 74 | 235 260 | 4.68 4.68 | | 247 | 191 |
| 30 | $G^{iBu}$ | $A^{Bz}$ | 66 | 235 263 276 | 4.69 4.58 4.60 | | 312 | 238 |
| 31 | $G^{iBu}$ | $G^{iBu}$ | 70 | 237 260 274 | 4.55 4.54 4.46 | | - | 222 |
| 14 | T | T | 67 | 233 266 | 4.58 4.47 | | | |
| 15 | $C^{Bz}$ | $C^{Bz}$ | 64 | 235 260 300 | 4.75 4.75 4.38 | | | |

[ ] = Shoulder; ::) = LiChrosorb Si 60-5, 25 cm, Chrompack; #) = LiChrosorb 10 RP 8, 25 cm, Chrompack.

under $N_2$ first at -20°C and then at room temp. and followed by high vacuum evaporation to remove all volatile components. The crude, but $^{31}$P-NMR spectroscopically pure material was then applied in a 1.6 molar excess to the base-protected 5'-O-dimethoxytrityl-2'-deoxyribonucleosides 1-4 to form the corresponding 2-(4-nitrophenyl) ethylphosphoromorpholidites 6-9 as colorless solids in 87-96 % isolated yields. Reaction of these intermediates towards formation

of phosphite internucleotide bonds with 3'-O-benzoyl-2'-deoxyribonucleosides (10-13) was achieved by 1H-tetrazole activation in acetonitrile followed by iodine oxidation to give the di-2'-deoxynucleoside phosphotriesters 16-31. Good yields have been obtained using a double-molar excess of the phosphoramidites 6-9 over the 5'-OH components 10-13. The intermediary 2-(4-nitrophenyl)ethyl phosphites can also be isolated and purified as shown for 14 and 15.

The characterization and structural proof of the newly synthesized compounds 16-31 was achieved by elementary analyses, UV- and $^{1}$H-NMR spectrometric means as well as HPLC investigations (Tab.). It was found that there is good separation into the two diastereoisomers in the system methylenechloride/methanol (97/3) except the $G^{iBu}$ containing components. The guanosine moiety causes much larger retention times even in such polar systems as methylenechloride/methanol (80/20), in which 31 is still not eluted. A much better comparison of the chromatographical behaviour was, however, encountered by reversed-phase chromatography on an HPLC-column LiChrosorb 10 RP 8 in methanol/water (85/15). Separation of the diastereomeric phosphotriesters cannot be achieved under these conditions, but there is good agreement that the presence of a polar amide group in the aglycon ring effects lower retention times as expected.

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