Tetrahedron Letters, Vol. 33, No. 48, pp. 7319-7322, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Synthesis and Selective Cleavage of an Oligodeoxynucleotide Containing a Bridged Non-Chiral Internucleotide 3'-Phosphoramidate Linkage

Matthias Mag, Rainer Schmidt and Joachim W. Engels*

Institut für Organische Chemie, Johann Wolfgang Goethe-Universität, Niederurseler Hang, 6000 Frankfurt am Main 50, FRG

Key Words: Staudinger reaction; dinucleoside-3'-phosphoramidate; oligodeoxynucleotide

Abstract: A self complementary oligodeoxynucleotide dodecamer containing an achiral bridged 3'-phosphoramidate linkage 3'-NH-P-O-5' has been prepared using the solid phase phosphoramidite procedure. Cleavage of the P-N bond can be accomplished selectively by mild acidolysis.

The synthesis of oligo(deoxy)nucleotides (ODNs) containing modified internucleotide phosphates has received considerable attention owing to their applicability for various biochemical purposes and because of their potential use as antiviral drugs.[1-3] A wide range of promising candidates has already been described in the literature. Analogs where one of the two bridging oxygen atoms in the phosphodiester linkage is replaced by sulfur or nitrogen are particularly attractive, but have received only little attention in the last decade.[4-17] These compounds are of interest for several reasons: (i) they are achiral at phosphorus and thus no diastereomers are created during their synthesis; (ii) they are electronically and sterically similar to the natural congener; (iii) they enable us to study the cleavage properties of the modified linkage by endo- and exonucleases; and (iv) these compounds are susceptible to specific cleavage exclusively at the point of modification under mild chemical conditions.

In analogy to our[15-17] and other[5-14] work on the synthesis of ODNs containing bridging 5'-phosphoramidate or 5' (3')-phosphorothioate linkages, we expected that also 3'-phosphoramidates are of interest. In this paper we wish to report the synthesis and selective cleavage of an ODN containing a **3'-NH-P-O-5'** linkage.

The synthesis of the 3'-phosphoramidate analog of TpdA **11a-d** useful for the synthesis of ODNs containing phosphoramidate linkages is outlined in **FIGURE 1** and **2**. One of the key intermediates, 5'-O-dimethoxytrityl-3'-azido-3'-deoxythymidine **4** (**FIGURE 1**), in the synthesis of **9a,b** is available from thymidine via a four-step reaction sequence in 70% overall yield.[18,19,20]

The second building block for the synthesis of the fully protected phosphoramidate dimer **9a,b** is the phosphite triester **8**. The preparation of **8** was started from N^6-benzoyl-2'-deoxyadenosine **5**.[18] This compound was transiently protected at the 5'-hydroxyl function by the dimethoxytrityl group in 82 % yield. After this regioselective reaction, this compound was allowed to react with 2 equivalents of tert.-butyldimethylsilylchloride (TBDMSCl) in the presence of imidazole at room temperature for 20 hours. The 5'-O-dimethoxytrityl group of **6** was removed with 80 % acetic acid at ambient temperature in 30 minutes to give **7** in 72 % yield. The phosphite triester **8** was then prepared in quantitative yield by treating the 3'-O-silylated nucleoside **7** with 1,1 equivalents of bis-methoxy-N,N-diisopropylaminophosphane in the presence of tetrazole (r. t., 2 h) and used without further purification.[15,16]

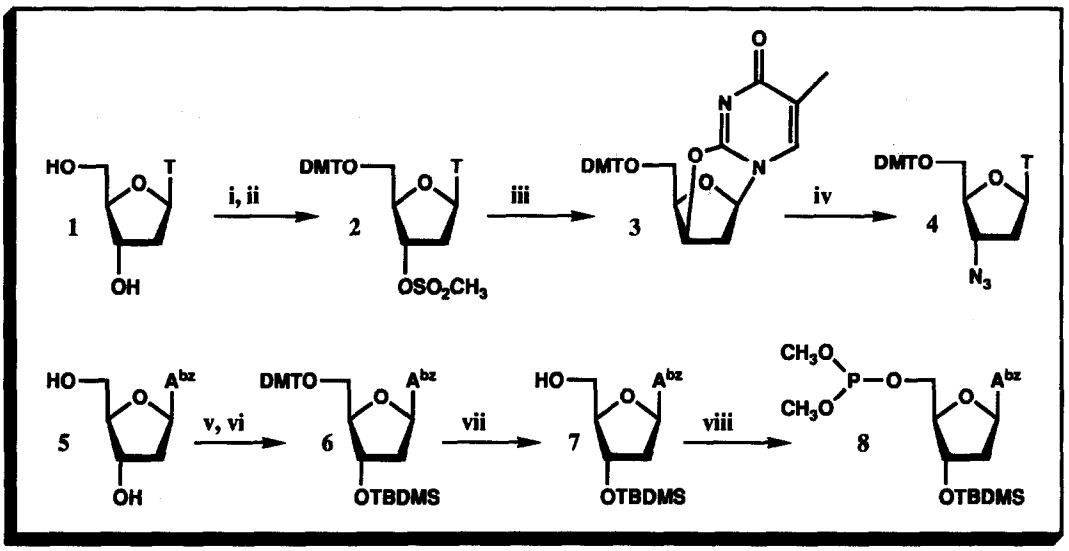

FIGURE 1: i, dimethoxytritylchloride, pyridine; ii, mesylchloride, pyridine; iii, potassium phthalimide, dimethylformamide; iv, LiN_3, dimethylformamide; v, dimethoxytritylchloride, pyridine; vi, TBDMSCl, pyridine; vii, 80 % acetic acid; viii, N,N-diisopropylamino-bis-methoxyphosphane and tetrazole in CH_2Cl_2/CH_3CN, 1:1

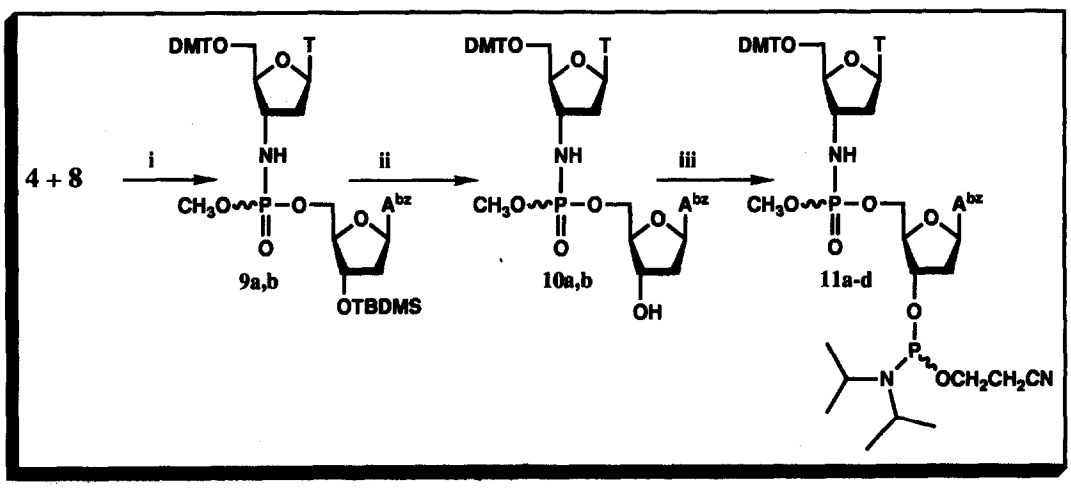

FIGURE 2: i, LiCl, pyridine; ii, tetrabutylammoniumfluoride, tetrahydrofuran; iii, N,N-diisopropylethylamine and rac-2-cyanoethoxy-N,N-diisopropylaminochlorophosphane in CH_2Cl_2/ CH_3CN, 1:1

The formation of the internucleotide phosphoramidate linkage 3'-NH-P-O-5' between the phosphite triester **8** and the 3'-azido nucleoside unit **4** to afford the dimer **9a,b** is illustrated in **FIGURE 2.** Therefore, the *in situ* prepared phosphite triester **8** (1,5 equivalents) in pyridine was treated with 1 equivalent **4** in the presence of 5 equivalents LiCl at ambient temperature for 16 hours. The initial phosphite imine in this **Staudinger** reaction is formed by the visible evolution of nitrogen, followed by

the conversion to the phosphoramidate by a **Michaelis-Arbuzov** type transformation which is enhanced by LiCl.[21-25] The reaction requires no further coupling or activating reagents. After flash chromatography and precipitation into cold n-pentane the desired dimer **9a,b** was obtained as a colorless amorphous solid in 79 % yield. The purity of this compound was checked by reverse phase HPLC and ^{31}P-NMR spectroscopy. The ^{31}P-NMR spectrum of **9a,b** shows two well resolved signals at 10,3 and 10,5 ppm in a ratio of nearly 1:1. This indicates that the reaction proceeds without diastereoselectivity. The constitution of the fully protected dinucleoside phosphoramidate **9a,b** was confirmed by means of homonuclear chemical shift correlation spectroscopy.

The desilylation was attempted with an excess of tetrabutylammoniumfluoride in tetrahydrofuran. The reaction proceeds only with 32 % yield. During the deprotection no observable degradation was observed by analytical TLC and after 45 minutes the reaction was complete. But after the usual work up with aqueous 5 % $NaHCO_3$ a number of degradation products were detectable. The crude product **10a,b** was purified by flash chromatography yielding a colorless powder after precipitation into cold n-pentane (^{31}P-NMR: 10,9 and 11,3 ppm). Subsequent phosphitylation of the 3'-hydroxyl dimer unit with rac-2-cyanoethoxy-N,N-diisopropylamino-chlorophosphane afforded the desired 3'-phosphoramidite building block **11a-d**. After flash chromatography and precipitation into cold n-pentane the compound could be isolated as a colorless powder in 88 % yield. Due to the introduction of a new chiral center by phosphitylation of the diastereomeric mixture **10a,b** the reaction could lead to $2^2 = 4$ possible diastereoisomers. To analyze this complex mixture and to prove the purity we recorded a ^{31}P-NMR spectrum in the proton decoupled mode. Theoretically one can observe two resonances for each of the four diastereoisomers. This could lead to 2 x 4 = 8 possible ^{31}P-NMR signals, four resonances located at the phosphoramidate region around 10 ppm and four resonances for the phosphite moiety around 150 ppm. The spectrum of the phosphitylated dimer **11a-d** shows a multiplet at 10,5 ppm and four singlets located at 149,66, 149,72, 149,78 and 149,95 ppm in a ratio of nearly 1:1:1:1. The spectrum makes the above assumption evident and confirms the structure of **11a-d**.

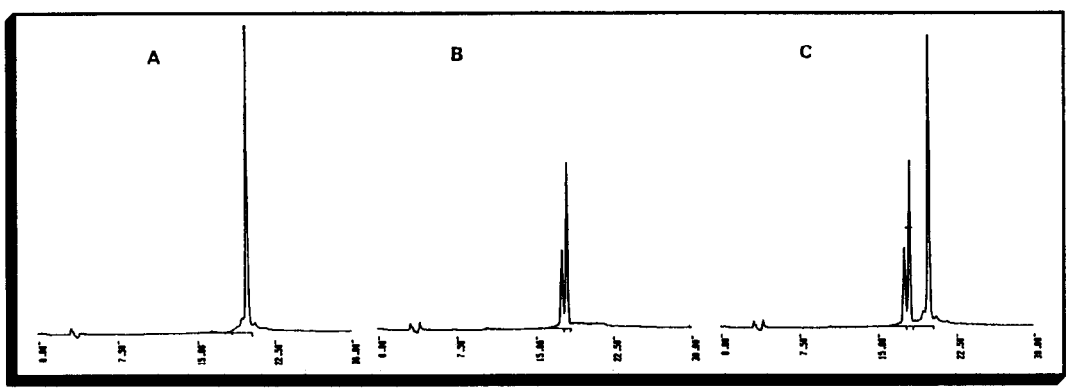

FIGURE 3: HPLC chromatograms: A) **12**; B) **12** + 80% CH_3COOH, 5h, r.t.; C) 1:1 mixture of A and B; Conditions: Nucleosil RP-C18 column (5µm, 4 x 250 mm), 5 --> 30% CH_3CN in triethylammonium acetate buffer (pH 7) in 30 min., 0,8 ml/min., 260nm

To study the usefulness of the new dimer phosphite unit **11a-d** the synthesis of dodecamer **12** was carried out using the dimer building block mentioned above (**12**: d[A G A G A T_{NH}pA T C T C T]). The synthesis was performed with a tenfold excess of **11a-d** in acetonitrile (1 µmol scale) which was

employed in the fifth reaction cycle instead of a monomeric phosphoramidite.[16,26] The modified building block **11a-d** was utilized under standard conditions except that the coupling time was increased from 30 to 240 s. The coupling efficiency of the internucleotide bond formation with this modified dimer building block was 96 %, the average coupling yield 97 % and the overall yield 72 % as determined by dimethoxytrityl color quantitation at 498 nm. These results are in agreement with those found for the incorporation of a dimer unit containing a 5'-phosporamid*a*te linkage by us[16] and others.[10]

Treatment with conc. ammonia removed the protected oligomer from the solid support and the protecting groups were removed at 55 °C during 24 hours. After evaporation to dryness the crude ODN was purified by preparative polyacrylamide gel electrophoresis.

To confirm the incorporation of the phosphoramid*a*te bond into the synthesized ODN **12** we treated 0,5 A_{260} units of the oligomer with 100 μl of 80 % acetic acid for 5 hours at room temperature. After this time the solution was evaporated to dryness, redissolved and analyzed by RP-HPLC (**FIGURE 3**). Whereas the naturally linked ODN was not susceptible to 80 % acetic acid the modified DNA fragment **12** was cleaved completely at the phosphoramid*a*te linkage under these conditions. The distinct pattern shows that the acid hydrolysis occurs selectively and does not harm the natural diester linkages.

The above procedure produces a **5'-phosphate** and a **3'-amino** oligomer analogous to the cleavage of ODNs containing a bridged 3'-phosphorothioate linkage as described by Cosstick et al.[11,12] Furthermore the method is complementary to that described by us[16] and others[10,13] for the incorporation of a **3'-O-P-NH-5'** linkage. The incorporation of a P-N linkage into a specific position of an ODN (primer) and the subsequent chemical cleavage can be applied for the nicking and manipulation of DNA, e. g. in a DNA probe based affinity assay.[27]

REFERENCES

1. Zon, G. (1988) Pharmaceutical Res., *5*, 539
2. Uhlmann, E. and Peymann, A. (1990) Chemical Rev., *90*, 544 and references cited therein
3. Hélène, C. and Toulmé, J. J. (1990) Biochimica et Biophysica Acta, *1049*, 99
4. Zielinski, W.S. and Orgel, L.E. (1987) Nucleic Acids Res., *3*, 1053
5. Letsinger, R.L., Hapke, B., Peterson, G.R. and Dumas, L.B. (1976) Nucleic Acids Res., *3*; 1053
6. Dolinnaya, N. G., Sokolova, N. I., Gryaznova, O. I. and Shabarova, Z. A. (1988) Nucleic Acids Res., *16*, 3721
7. Cook, A.F. (1970) J. Am. Chem. Soc., *92*, 190
8. Chladek, S. and Nagyvary, J. (1972) J. Am. Chem. Soc., *94*, 2079
9. Bogachev, V.S., Kumarev, V.P. and Rybakov, V.N. (1986) Bioorg. Khim., *12*, 133
10. Gryaznov, S.M. and Azhaev, A.V. (1989) Bioorg. Khim., *15*, 994
11. Cosstick, R. and Vyle, J.S. (1989) Tetrahedron Lett., *30*, 4693
12. Cosstick, R. and Vyle, J.S. (1989) Nucleic Acids Res., *18*, 829
13. Bannwarth, W. (1988) Helv. Chem. Acta, *71*, 1517
14. Sund, C. and Chattopadhyaya, J. (1989) Tetrahedron, *45*, 7523
15. Mag, M. and Engels, J.W. (1988) Nucleosides and Nucleotides, *7*, 725
16. Mag, M. and Engels, J.W. (1989) Nucleic Acids Res., *17*, 5973
17. Mag, M., Lüking, S. and Engels, J.W. (1991) Nucleic Acids Res., *19*, 1437
18. Gait, M.J. (1984) Oligonucleotide Synthesis, edited by M.J. Gait, IRL Press Oxford
19. Zuckermann, R., Corey, D. and Schultz, P. (1987) Nucleic Acids Res., *15*, 5305
20. Horwitz, J.P., Chua, J. and Noel, M. (1964) J. Org. Chem., *29, 1772*
21. Freist, W., Schattka, K., Cramer, F. and Jastorff, B. (1972) Chem. Ber., *105*, 991
22. Letsinger, R.L. and Heavner, G.A. (1975) Tetrahedron Lett., *2*, 147
23. Hata, T., Yamamoto, I. and Sekine, M. (1976) Chemistry Letters, 601
24. Nielson, J. and Caruthers, M.H. (1988) J. Am. Chem. Soc., *110*, 6275
25. Gibbs, D.E. (1977) Tetrahedron Lett., *8*, 679
26. Kumar, G. and Poonian, M.S. (1984) J. Org. Chem., *49*, 4905
27. Cosstick, R. and Carr, F. (Scotgen Ltd.) Brit. UK Pat. Appl. GB 2,236,852

(Received in Germany 20 July 1992)