Pergamon

Tetrahedron Letters, Vol. 38, No. 13, pp. 2241-2244, 1997

0040-4039/97 $17.00 + 0.00

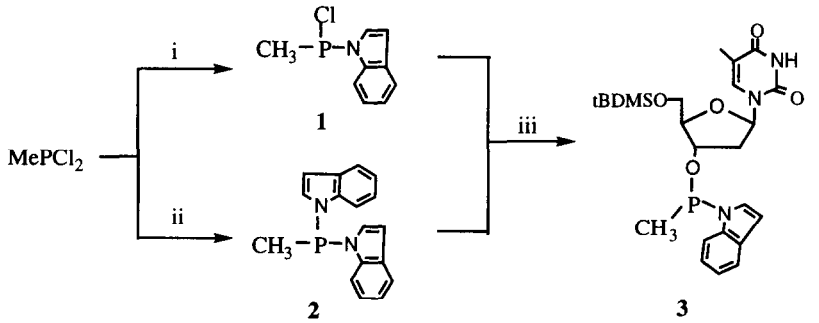
PII: S0040-4039(97)00333-X

Indole: A Novel Leaving Group in the Synthesis of Oligonucleoside Methylphosphonates

Jian-Chao Wang and George Just*
Department of Chemistry, McGill University, Montreal, Canada H3A 2K6

Abstract: A P^{III} coupling reagent 3 containing indole group is described. Its usefulness is demonstrated by the synthesis of dinucleoside methylphosphonates and methylthiophosphonates. © 1997 Elsevier Science Ltd.

The oligonucleoside methylphosphonates (MP) show significant potential for antisense therapy.[1] The internucleoside methylphosphonate linkage was first prepared by using activated nucleoside methylphosphonates,[2-7] but the coupling reactions were slow. Later, methylphosphoramidite approaches were utilized.[8,9,10] Unfortunately, all these methods yield a mixture of diastereoisomers. Stec and his coworkers have described a route using tetracoordinate phosphorus to synthesize stereoregular MP linkages by using p-nitrophenoxy[11,12] and methylselenenyl[13] as the leaving group, but the coupling yields were low, and each step required chromatographic purification of the MP products. We wish to report a very efficient synthesis of 3',5'-dithymidine methylphosphonate and its sulfur analogue with a novel internucleoside coupling procedure based on tricoordinate phosphorus reagent **3**.



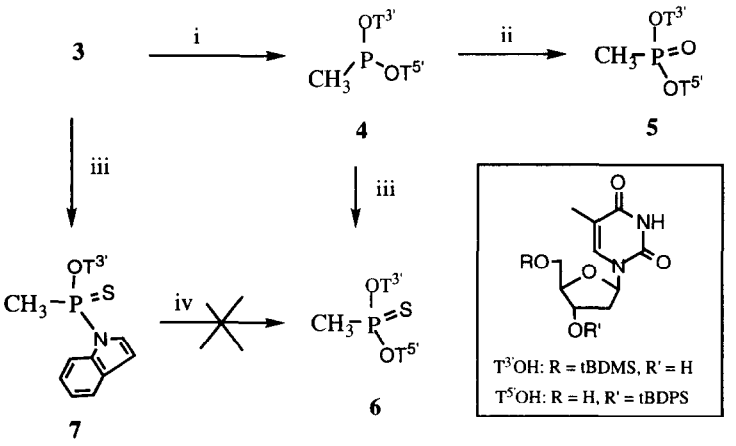
Scheme 1

i) Indole (1 eq.), Et_3N/CH_2Cl_2. ii) Indole (2 eq.), Et_3N/CH_2Cl_2. iii) 5'-O-tBDMS-thymidine.

The coupling reagent **3** was easily prepared by a one-flask procedure, shown in Scheme 1. Dichloromethylphosphorine reacted with one equivalent of indole in the presence of triethylamine to give quantitatively methyl indolphosphorochloridite **1**, which was then reacted with one equivalent of 5'-O-tBDMS-thymidine ($T^{3'}OH$). After filtering out triethylammonium chloride and drying in *vacuo* , two diastereoisomers **3a**, **3b** were obtained in quantitative yield as established by ^{31}P NMR. The product did not need further purification and could be stored under argon for a long time. When dichloromethylphosphorine reacted with

two equivalents of indole, methyl diindolphosphorine **2** was obtained, in which one indole group could be replaced by an alcohol in the presence of triethylamine to form **3**, while the indole group in **3** could not be replaced by alcohols using triethylamine as a base.

The coupling reaction of **3a**, **3b** with 3'-O-tBDPS-thymidine ($T^{5'}OH$) was done in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). By using two equivalents of DBU, the coupling reaction was completed to form two diastereoisomers of methylphosphinite **4a**, **4b** within 0.5 hour at 50^0C. Increasing the amount of DBU accelerated the coupling reaction. The methylphosphinite **4a**, **4b** could be efficiently oxidized to form methylphosphornate **5a**, **5b** by iodine/water.[10] With the Beaucage's reagent,[14] **4a**, **4b** led to the methylthiophosphonates **6a**, **6b**. The two diasteroisomers **5a**, **5b** and **6a**, **6b** could be separated by flash chromatography on silica gel.[15,16] The whole procedure starting from dichloromethylphosphorine to phosphonate **5** or **6** was done in one flask with an overall yield above 91%.



Scheme 2

i) $T^{5'}OH$, DBU. ii) 0.1M iodine in THF-pyridine-H_2O (4:3:3 v/v). iii) Beaucage's reagent. iv) $T^{5'}OH$, DBU or t-BuMgCl.

The coupling step is stereoselective. It is difficult to separate two diastereoisomers of **3a** and **3b** by silica gel chromatography because **3a** and **3b** are easily oxidized on silica gel. By using a TLC plate (Kieselgel 60 F_{254} glass backed plates, 0.5 mm thickness), a small amount of one diastereoisomer **3a** was separated and reacted with 5'-O-tBDMS-thymidine in the presence of DBU to afford only one diastereoisomer **4a**. Figure 1 shows the appropriate ^{31}P NMR spectra. Treatment of **3a**, **3b** with Beaucage's reagent gave two diastereoisomers **7a**, **7b**, ^{31}P NMR (109.3 MHz, $CDCl_3$): 82.83 ppm, 82.94 ppm (1:1). Unlike the p-nitrophenoxy group,[10] the indole group in the pentavalent phosphorus compound **7** was stable, and could not be replaced by alcohols in the presence of DBU or by a Grignard reagent.

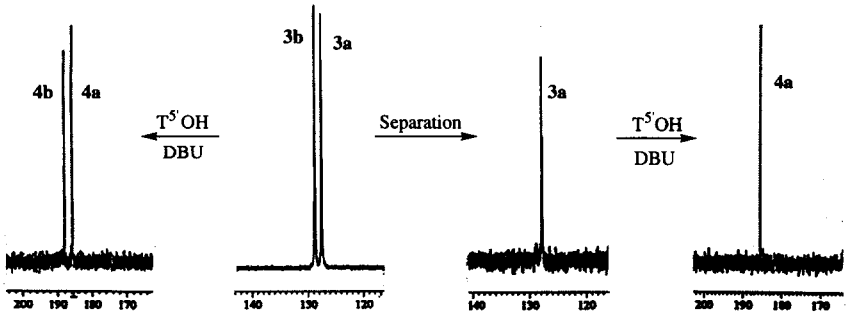


Figure 1. The ^{31}P NMR spectra of **3** and **4**.

In conclusion, we described an efficient way to synthesize dinucleoside methylphosphonates, in which the coupling step of **3** to **4** is stereoselective.

Coupling reagent **3**. Route A: To 50 mg of indole (1 eq.) and 132 µl triethylamine (2.2 eq.) in 2 ml dry dichloromethane was added 42.5 µl dichloromethylphosphorine (1 eq., ^{31}P NMR 196.2 ppm) at 0°C. After a few minutes, a new peak at 112.2 ppm appeared corresponding to methyl indolphosphorochloridite **1**. Then 152 mg of 5'-O-tBDMS-thymidine (1 eq.) in 0.5 ml dry dichloromethane was added. The reaction was completed after a few minutes, and two diastereoisomers **3a**, **3b** were quantitatively formed as established by ^{31}P NMR (128.58 ppm, 129.50 ppm, 1:1). This solution was used for the next step.

Route B: To 100 mg of indole (2 eq.) and 132 µl triethylamine (2.2 eq.) in 2 ml dry dichloromethane was added 42.5 µl dichloromethylphosphorine (1 eq.) at 0°C. Immediately, methyl diindolphosphorine **2** was formed (^{31}P NMR, 47.1 ppm). Then 5'-O-tBDMS-thymidine (1eq.) in 0.5 ml dry dichloromethane was added. The mixture was kept at 50°C for 3 hours to provide **3**. This solution was used for the next step. The excess indole did not interfere with the following reactions.

Methylphosphinite **4**. To either of the above solutions was added 206 mg of 3'-O-tBDPS-thymidine (1 eq.) in 1 ml dry dichloromethane and 129 µl DBU (2 eq.). The mixture was kept at 50°C for 0.5 hour to form two diastereoisomers **4a**, **4b** as established in ^{31}P NMR (185.45 ppm, 187.70 ppm, 1:1).

Methylphosphonate **5**. The solution of **4** prepared above was treated with 0.1M of iodine in THF-pyridine-H_2O (4:3:3 v/v) for 5 minutes. Flash chromatography on silica gel afforded the two methylphosphonates **5a** and **5b.**

Acknowledgments

We wish to thank the Natural Science and Engineering Research Council of Canada and ISIS Pharmaceuticals, Carlsbad, CA, for the generous financial support.

References and notes

1. Murray, J. A. H., Antisense RNA and DNA, John Wiley and Sons, Inc. **1992**, pp.241-253.
2. Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K.; Ts'o, P. O. P., *Biochemistry* **1979**, *18*, 5134-5143.

3. Agarwal, K. L.; Riftina, F., *Nucleic Acids Res.* **1979**, *6*, 3009-3024.
4. Miller, P. S.; Agris, C. H.; Blandin, M.; Murakami, A.; Reddy, P. M.; Spitz, S. A.; Ts'o, P. O. P., *Nucleic Acids Res.* **1983**, *11*, 5189-5204.
5. Miller, P. S.; Agris, C. H.; Murakami, A.; Reddy, P. M.; Spitz, S. A.; Ts'o, P. O. P., *Nucleic Acids Res.* **1983**, *11*, 6225-6242.
6. Marugg, J. E.; Vroom, E.; Dreef, C. E.; Tromp, M.; Marel, G. A.; Boom, J. H., *Nucleic Acids Res.* **1986**, *14*, 2171-2185.
7. Miller, P. S.; Reddy, M. P.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Agris, C. H., *Biochemistry* **1986**, *25*, 5092-5097.
8. Jager, A.; Engles, J., *Tetrahedron Lett.* **1984**, 25, 1437-1440.
9. Agrawal, S.; Goodchild, J., *Tetrahedron. Lett.* **1987**, *28*, 3539-3542.
10. Helinski, J.; Dabkowski, W.; Michalshi, J., *Tetrahedron. Lett.* **1991**, *32*, 4981-4984.
11. Lesnikowski, Z. J.; Wolkanin, P. J.; Stec, W. J., *Tetrahedron. Lett.* **1987**, *28*, 5535-5538.
12. Lesnikowski, Z. J.; Jaworska, M.; Stec, W. J., *Nucleic Acids Res.* **1988**, *16*, 11675-11689.
13. Wozniak, L. A.; Pyzowski, J.; Wieczorek, M.; Stec, W. J., *J. Org. Chem.* **1994**, *59*, 5843-5846.
14. Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L., *J. Am. Chem. Soc.* **1990**, *112*, 1253-1254.
15. **5a**, ^{31}P NMR (109.3 MHz, $CDCl_3$): 33.29 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): 9.73 (br, s, 2H, NH-T$^{3'}$, NH-T$^{5'}$), 7.31-7.62 (m, 12H, $C_6H_5SiC_6H_5$, H-6-T$^{3'}$, H-6-T$^{5'}$), 6.37 (m, 1H, H-1'-T$^{5'}$), 6.27 (dd, ^{3}J = 9.0 Hz, 5.0 Hz, 1H, H-1'-T$^{3'}$), 4.95 (m, 1H, H-3'-T$^{3'}$), 4.30 (m, 1H, H-3'-T$^{5'}$), 4.12 (m, 1H, H-4'-T$^{3'}$), 4.08 (m, 1H, H-4'-T$^{5'}$), 3.88, 3.57 (m, 2H, H-5', H-5''- T$^{5'}$), 3.82 (m, 2H, H-5', H-5''-T$^{3'}$), 2.33, 1.81 (m, 2H, H-2', H-2''-T$^{5'}$), 2.30, 1.98 (m, 2H, H-2', H-2''-T$^{3'}$), 1.90 (s, 3H, CH_3C-5-T$^{5'}$), 1.86 (s, 3H, CH_3C-5-T$^{3'}$), 1.37 (d, $^{3}J_{P\text{-}H}$ = 17.5 Hz, 3H, CH_3P), 1.06 (s, 9H, C(CH_3)3-T$^{5'}$), 0.88 (s, 9H, C(CH_3)3-T$^{3'}$), 0.069 (d, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 179.63, 164.13, 150.61, 150.55, 135.77, 135.75, 135.48, 134.89, 133.05, 132.78, 130.28, 130.25, 128.07, 111.34, 111.31, 86.00, 85.39, 85.30, 84.58, 73.09, 65.00, 64.91, 63.22, 42.92, 40.48, 26.89, 25.95, 19.06, 18.33, 12.58, 12.42, 10.60, -5.33, -5.44. **5b**, m.p. 94-95^{0}C. ^{31}P NMR (109.3 MHz, $CDCl_3$): 34.17 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): 8.66 (br, s, 1H, NH-T$^{3'}$), 8.59 (br, s, 1H, NH-T$^{5'}$), 7.22-7.65 (m, 12H, $C_6H_5SiC_6H_5$, H-6-T$^{3'}$, H-6-T$^{5'}$), 6.39 (m, 1H, H-1'-T$^{5'}$), 6.30 (dd, ^{3}J = 9.5 Hz, 5.0 Hz, 1H, H-1'-T$^{3'}$), 4.96 (m, 1H, H-3'-T$^{3'}$), 4.28 (m, 1H, H-3'-T$^{5'}$), 4.06-4.14 (m, 2H, H-4'-T^{3}, H-4'-T$^{5'}$), 3.76-3.90 (m, 4H, H-5', H-5''- T$^{5'}$, H-5', H-5''-T$^{3'}$), 2.40, 2.08 (m, 2H, H-2', H-2''-T$^{3'}$), 2.35, 1.84 (m, 2H, H-2', H-2''-T$^{5'}$), 1.91 (s, 3H, CH_3C-5-T$^{5'}$), 1.88 (s, 3H, CH_3C-5-T$^{3'}$), 1.42 (d, $^{3}J_{P\text{-}H}$ = 17.5 Hz, 3H, CH_3P), 1.08 (s, 9H, C(CH_3)3-T$^{5'}$), 0.90 (s, 9H, C(CH_3)3-T$^{3'}$), 0.10 (d, 6H, CH_3SiCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 163.97, 163.91, 150.60, 150.47, 135.77, 135.73, 134.89, 132.92, 132.76, 130.34, 130.28, 128.09, 128.06, 111.46, 111.31, 86.06, 85.31, 85.15, 84.69, 72.81, 65.02, 64.93, 63.24, 40.39, 39.65, 26.89, 25.97, 19.06, 18.34, 12.58, 12.52, 10.59, -5.35, -5.40.
16. Compounds **6a**, **6b** were characterized by ^{1}H NMR, COSY and ^{13}C NMR.

(*Received in USA* 20 *January* 1997; *accepted* 13 *February* 1997)