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A Stereoselective Synthesis of Dinucleotide Phosphorothioate Triesters through a Chiral Indol-oxazaphosphorine Intermediate

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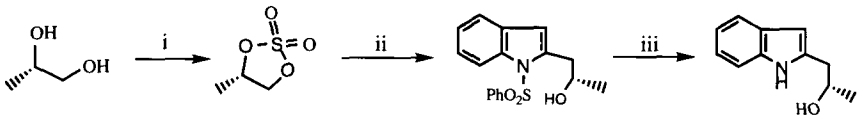
Abstract: *(S)-1-(indol-2-yl)-propan-2-ol was used as a chiral auxiliary to form a dinucleotide phosphorothioate triester in 97% ee.* © 1997, Elsevier Science Ltd. All rights reserved.

Oligonucleotide phosphorothioates (PS-Oligos) have attracted much attention due to their therapeutic potential.[1] A still unsolved and often unappreciated problem concerning the use of PS-Oligos in the antisense strategy is their polydiastereoisomerism. PS-Oligos, currently employed in clinical studies and biological evaluations, are obtained as mixtures of 2^n diastereomers, where n is equal to the number of internucleotidic phosphorothioate linkages. To date the most useful stereoselective synthesis of PS-Oligos has been described by Stec and coworkers.[2,3] Their methods suffer from the fact that the chiral precursors have to be separated chromatographically. Recently, we developed synthesis for chiral cyclic phosphoramidites, which were obtained from xylose and could be used without purification for the preparation of diastereomerically enriched PS-Oligos.[4,5] We also prepared chiral bicyclic imidazo-oxazaphosphorines, which were obtained with high de from a chiral precursor by a simple equilibration. Although the latter gave PS-Oligos with excellent de's, they were too reactive to be used routinely.[6] We here describe the use of indole, pKa 16.97, instead of imidazole, pKa 14.10, in the stereoselective synthesis of phosphorothioate triesters.

We chose (S)-1-(indol-2-yl)-propan-2-ol **1** as a chiral auxiliary. It was synthesized from the sulfate[7] of (S)-propanediol and the anion of 1-phenylsulfonyl-indole,[8] as outlined in scheme 1.



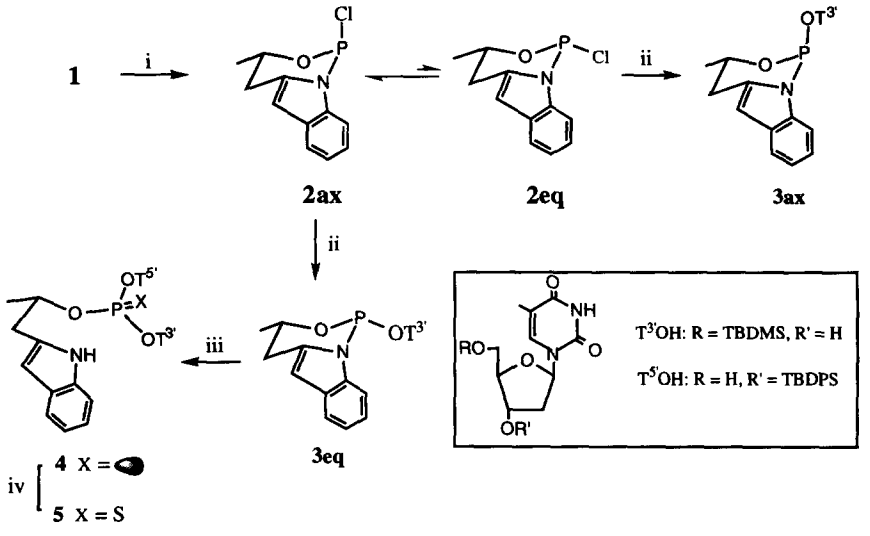
1

Scheme 1

i) a. $SOCl_2$, CCl_4, 60°C. b. $NaIO_4$, $RuCl_3 3H_2O$, CH_3CN/H_2O, 25°C, 98%. ii) 1-Phenylsulfonyl-indole,n-BuLi, -78°C-25°C, overnight, then add 20% H_2SO_4 and stir for 3 hours, 87%. iii) KOH, CH_3OH/H_2O (3:1), reflux, 100%.

Equimolar acetonitrile solutions of **1** and PCl_3 were allowed to react at 0°C under argon and the reaction was followed by ^{31}P NMR. After a few minutes, the total disappearance of the peak corresponding to PCl_3 at 221 ppm was observed, and several peaks appeared around 140 - 150 ppm. The mixture was warmed up to 60°C. The warming was continued (about 10 hours) until the ^{31}P NMR showed a major peak at 144 ppm,

which indicated the formation of phosphorochloridite **2**. It probably exists as a rapidly equilibrating mixture of **2ax** and **2eq**, in which **2ax** predominates (*vide infra*). The mixture was cooled to 0°C and a solution of 5'-O-tBDMS-thymidine in CH_2Cl_2 was added. Two peaks were observed within 0.5 h, a major one at 120.47 ppm and a minor one at 120.36 ppm, corresponding to the formation of the two diastereoisomers **3eq** and **3ax**.[10] The ratio of two diastereoisomers of **3** was affected by the temperature at which 5'-O-tBDMS-thymidine was added. At 20-60°C, the ratio was 7:1; at lower temperature (0 – -78°C), the ratio increased to 9:1.



Scheme 2

i) PCl_3, CH_3CN/Et_3N, 0°C - 60°C. ii) T3'OH. iii) T5'OH, DBU. iv) Beaucage's reagent.

In our previous studies,[4,5,6] the two diastereoisomers at phosphorus of phosphoramidites **A** and imidazo-oxazaphosphorine **B** could be equilibrated by heating their solutions containing triethylammonium chloride to form the thermodynamically more stable one which the R group was at the axial position. In marked contrast, **3ax**, **3eq** could not be equilibrated by heating in the presence of either acid or base. Diastereomers **3ax**, **3eq** could not be separated by flash chromatography on silica gel.

A **B**

The coupling step of **3** with 3'-O-tBDPS-thymidine was done in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). The major diastereoisomer **3eq** reacted much faster with 3'-O-tBDPS-thymidine than the minor axially substituted isomer **3ax**. By using 1 eq. of DBU and 1 eq. of 3'-O-tBDPS-thymidine, 95% of **3eq** was converted to phosphite triester **4** after five hours at 50°C, while **3ax** almost did not

react. Figure 1 shows the ^{31}P NMR of the starting material **3** and the ^{31}P NMR of the reaction mixture of **3** with thymidine after 17 hrs at room temperature. After filtration through a short silica gel column to remove DBU, triester **4** was treated with Beaucage's reagent[9] to give a 73:1 mixture of phosphorothioates **5**,[11] in which the major isomer most probably has the Rp configuration, ^{31}P NMR 66.76 ppm (major) and 66.59 ppm (minor). The chiral auxiliary **1** could not be removed with 28% ammonium hydroxide.

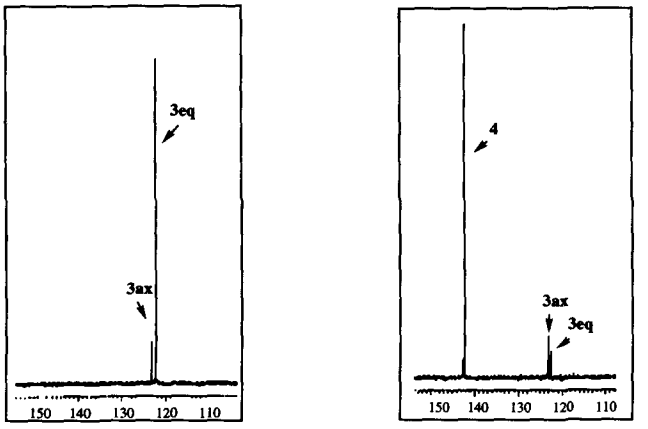


Figure 1

We had shown that the thermodynamically more stable axially substituted phosphoramidites **A** reacted much more slowly than their equatorially substituted isomers.[4] This is probably also true for the cyclic indole derivatives **2** and **3**. The results are best explained by postulating that the displacement leading from **2** to **3**, and **3** to **4** proceed with inversion. The rapidly equilibrating mixture of slow reacting **2ax** and faster reacting **2eq** (ratio ~ 99:1 ?) provides a 7-9:1 mixture of non-equilibrating fast reacting **3eq** and slow reacting **3ax**. The fast reacting major indole derivative **3eq** and its slow reacting minor isomer **3ax** are then transformed with inversion to provide **4** as a mixture of diastereomers, in which one isomer predominates.

In conclusion, we report that indol-oxazaphosphorine of type **2** are useful intermediates for the stereoselective synthesis of chiral phosphorothioate triesters. Further studies are in progress to develop similar chiral auxiliaries which can be easily removed.

Acknowledgments

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10. **3**. m.p. 80-82^{0}C. ^{31}P NMR (202.3 MHz, $CDCl_3$): **3ax**, 121.56 (12.4%). **3eq**, 120.67 (87.6%). **3eq** ^{1}H NMR (500 MHz, $CDCl_3$): 8.81 (br s, 1H, NH), 7.39 (s, 1H, H-6), 7.54, 7.17 (m, 4H, C_6H_4), 6.36 (dd, 1H, ^{3}J = 9.0 Hz, ^{3}J = 5.5 Hz, H-1'), 6.33 (s, 1H, C=CH-Ph), 4.72 (m, 1H, H-3'), 4.41 (m, 1H, CHOP), 3.94 (m, 1H, H-4'), 3.58 (m, 1H, H-5'), 3.06 - 3.10 (m, 3H, H-5'', CH_2), 2.36 (m, 1H, H-2'), 1.97 (m, 1H, H-2''), 1.87 (s, 3H, CH_3C-5), 1.48 (d, 3H, ^{3}J = 5.5 Hz, CH_3), 0.84 (s, 9H, $SiC(CH_3)_3$), -0.05 (d, 6H, $Si(CH_3)_2$). ^{13}C NMR (67.9 MHz, $CDCl_3$): 163.7 (C-4), 150.3 (C-2), 137.6, 129.8, 122.2, 121.5, 120.4, 111.1 (C_6H_4), 136.4 (**C**CHPh), 135.2 (C-6), 110.6 (C-5), 103.2 (**C**CHPh), 86.2 (C-4'), 86.1 (CHOP), 84.8 (C-1'), 73.7 (C-3'), 71.5 (C-5'), 62.9 (C-2'), 26.0 (CH2), 25.9 ($SiC(\mathbf{C}H_3)_3$), 23.0 ($SiC(\mathbf{C}H_3)_3$), 18.3 (CH_3), 12.6 ($\mathbf{C}H_3$C-5), -5.54, -5.77 (CH_3SiCH_3). HRMS (FAB, M+H): Cal. 560.234578, found 560.234590.
11. **5**. m.p. 115-116^{0}C. ^{31}P NMR (202.3 MHz, $CDCl_3$): 66.76 (98.65%), 66.59 (1.35%). ^{1}H NMR (500 MHz, $CDCl_3$): 9.93 (s, 1H, NH), 9.31 (s, 1H, NH-3-T$^{5'}$), 8.85 (s, 1H, NH-3-T$^{3'}$), 7.62 - 6.93 (m, 16H, $Si(C_6H_5)_2$, C_6H_4, H-6- T^3, H-6- T$^{5'}$), 6.46 (dd, 1H, ^{3}J = 8.0 Hz, ^{3}J = 6.0 Hz, H-1'- T$^{5'}$), 6.26 (s, 1H, CH-Ph), 6.05 (dd, 1H, ^{3}J = 9.2 Hz, ^{3}J = 5.5 Hz, H-1'- T^3), 4.92 (m, 1H, CHOP), 4.76 (m, 1H, H-3'- T^3), 4.31 (m, 1H, H-3'- T$^{5'}$), 4.03 (m, 1H, H-4'- T$^{5'}$), 3.82 (m, 1H, H-4'- T^3), 3.80, 3.50 (m, 2H, H-5', H-5''- T$^{5'}$), 3.67, 3.58 (m, 2H, H-5', H-5''- T^3), 3.00 (m, 2H, CH_2), 2.31 (m, 1H, H-2'- T$^{5'}$), 1.94 (s, 3H, CH_3C-5- T$^{5'}$), 1.90 (s, 3H, CH_3C-5- T^3), 1.85 (m, 1H, H-2''- T$^{5'}$), 1.60 (m, 1H, H-2'- T^3), 1.26 (d, 3H, ^{3}J = 6.0 Hz, CH_3), 1.14 (m, 1H, H-2''- T^3), 1.80 (s, 9H, $SiC(CH_3)_3$- T$^{5'}$), 0.89 (s, 9H, $SiC(CH_3)_3$- T^3), 0.07 (d, 6H, $Si(CH_3)_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$): 163.84, 163.80 (C-4- T^3. C-4- T$^{5'}$), 150.74, 150.34 (C-2- T^3, C-2- T$^{5'}$), 135.52, 135.49, 135.25, 134.56, 134.16, 132.73, 132.55, 130.15, 130.05, 128.48, 127.91, 127.85, 120.99, 119.59, 119.34, 111.24, 110.41 ($C_6H_5SiC_6H_5$, C_6H_5NC, C-6- T^3, C-6- T$^{5'}$), 100.69 (PhCH), 85.29, 85.17 (C-4'- T^3, C-4'- T$^{5'}$), 84.87 (C-1'- T$^{5'}$), 84.27 (C-1'- T^3), 79.70 (C-3'-T^3), 76.82 (CH), 73.31 (C-3'- T$^{5'}$), 66.83 (C-5'- T$^{5'}$), 63.01 (C-5'- T^3), 40.17 (C-2'- T$^{5'}$), 37.59 (C-2'- T^3), 36.00 (CH_2), 26.67 ($C(CH_3)_3$- T$^{5'}$), 25.77 ($C(CH_3)_3$- T^3), 21.24 (CH_3), 18.81, 18.15 (SiC- T^3, SiC- T$^{5'}$), 12.42, 12.33 (CH_3-T$^{3'}$, CH_3-T$^{5'}$), -5.61, -5.56 (CH_3SiCH_3). MS (FAB, M+H): 1072.

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