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A Stereoselective Synthesis of Dinucleotide Phosphorothioates, using Chiral Phosphoramidites as Intermediates

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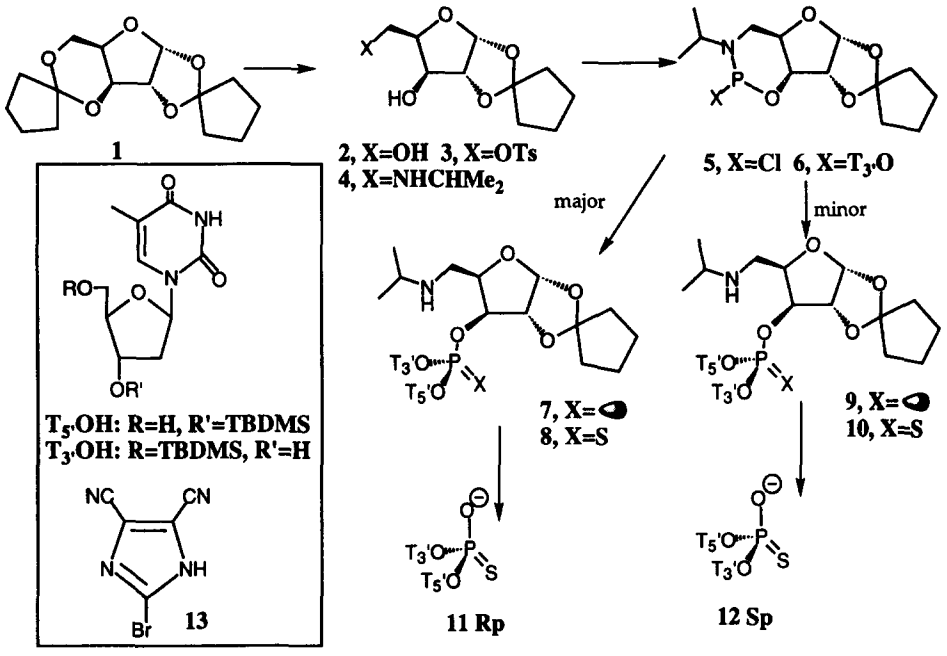
Abstract: 1,2-Di-O-cyclopentylidene-5-isopropylamino-D-xylofuranose **4** and its enantiomer *ent*-**4** were used as chiral auxiliaries to form, respectively, Rp and Sp dinucleotide phosphorothioates **11** and **12** in 98% diastereomeric excess, using phosphoramidite methodology and 2-bromo-4,5-dicyanoimidazole as catalyst.

In the preceding article,[1] we demonstrated that phosphoramidites derived from chiral 3-aminoalcohols could be obtained as single diastereomers without extensive purification, and that they could be transformed stereoselectively to phosphite triesters in ratios of diastereomers ranging from 3:1 to 50:1 for large and small nucleophiles, respectively. The chiral auxiliary used did not permit its removal at the end of a sequence leading to a phosphorothioate, and did not provide a diastereomeric excess (de) large enough to make it useful in the automated synthesis of oligophosphorothioates. In this paper, we describe chiral auxiliaries which are inexpensive, readily available as both enantiomers, and which contain a masked aldehyde group β to the hydroxyl group, thus permitting a base or acid-catalysed elimination of the auxiliary.

1,2-Di-O-isopropylidene-D-xylose seemed to fulfill the requirements. In the event, the harsh acidic conditions to remove the chiral auxiliary were not compatible with the acid-lability of the sulfur analog of the dinucleotide (see **8**), and the synthesis was carried out with the more acid-labile cyclopentylidene derivative of D-xylose.

Reaction of D-xylose with a mixture of cyclopentanone and trimethyl orthoformate in the presence of p-toluene sulfonic acid in p-dioxane [2] gave 1,2-di-O-3,5-di-O-dicyclopentylidene-D-xylofuranose **1**. Stirring **1** in acetic acid-water for 3 h at RT gave diol **2**. Selective tosylation was achieved by treating a 0.1M pyridine solution of **2** with a 40% excess of p-toluenesulfonyl chloride. Tosylate **3** was then heated in a ten-fold excess of isopropylamine at 55°C overnight to provide amine **4**, m.p. 44-45°C, $[\alpha]^{20}_{D}$=31.06° (c=2,$CHCl_3$). The overall yield for the transformation **1** to **4** was ~60%.

The formation of phosphochloridite **5** was carried out in a scrupulously dried NMR tube by first syringing in 0.11 mmole of PCl_3, followed by 0.25 ml of $CDCl_3$. After cooling to 0°C, a solution of 0.1 mmole of amino alcohol **4** in 0.35 ml $CDCl_3$ containing 0.22 mmol of NEt_3 was added. The NMR tube was then cooled to -78°C, sealed and warmed up to 40°C. The warming was continued until the ^{31}P NMR showed a single peak at δ 148.42 ppm. To the solution obtained was added slowly at 0°C 5'-O-t-butyldimethylsilyl thymidine (**T3'OH**, 0.1 mmole) in 0.45 ml of $CDCl_3$ containing 0.1 mmol of NEt_3. The NMR tube was cooled to -78°C, resealed and warmed at 50°C until the ^{31}P NMR showed a single peak at 130.14 ppm. The

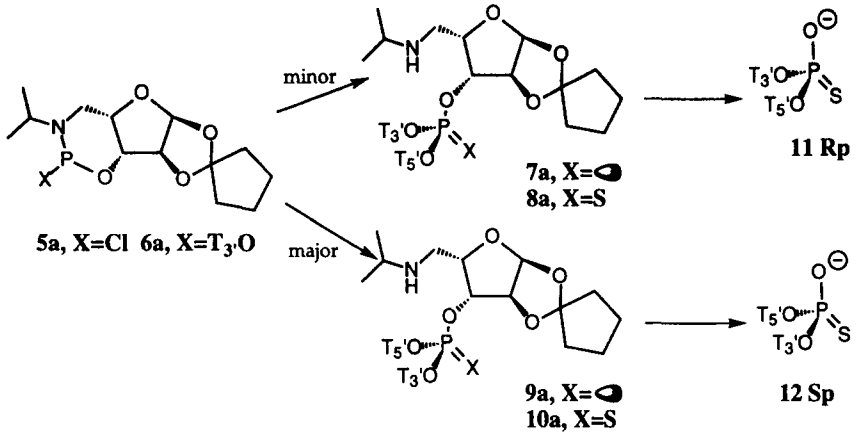


reaction mixture was diluted with EtOAc and washed with aq. $NaHCO_3$. Chromatography gave phosphoramidite **6**.[3a] The reaction could be scaled up to obtain **6** on a large scale.

Phosphoramidite **6** (15 mg), 3'-O-t-butyldimethylsilyl thymidine (**T_5'OH**, 1.2 eq) and 2-bromo-4,5-dicyanoimidazole **13** (2.0eq) were dried in a NMR tube, and 0.5 ml of dry acetonitrile was added under a nitrogen atmosphere. After a few minutes, ^{31}P NMR indicated the complete disappearance of **5**, and the appearance of phosphite triesters **7** and **9** in a ratio of 6:1, as established by ^{31}P NMR (143.76 and 142.55 ppm for major and minor isomer, respectively). Without purification, the mixture of triesters was treated with Beaucage's reagent [4] to give a 6:1 mixture of phosphorothioates **8** and **10**, ^{31}P NMR 68.23 and 68.43 ppm. When a similar reaction was carried out at 0°C for 4 hours and at -15°C for 6 hours, using deuteriochloroform as the solvent, the ratio of **7** to **9** increased to 40:1 and 68:1, respectively. In each case, the ratios were established by ^{31}P NMR. Chromatography of the reaction mixture obtained at -15°C only provided one isomer **8**.[5a] After hydrolysis of **8/10** with 70% trifluoroacetic acid at RT, the phosphorothioate dinucleotide **11** (^{31}P NMR 58.64ppm) and **12** (^{31}P NMR 58.57ppm) were obtained with Rp : Sp diastereomer ratio being the same as the ratio of **8** to **10** (Yield: 85%. The H-NMR and MS confirmed the structures of **11** and **12**). The absolute configuration of these final products was based on those ^{31}P NMR spectra and comparison with the data presented in the literature.[6]

In a parallel run, L-xylose was transformed to the enantiomer *ent*-**4**, m.p.39-41°C, $[a]^{20}_D$=-31.37° (c=2,$CHCl_3$). In a series of reactions identical to those described, *ent*-**4** was converted via phosphochloridite **5a** (^{31}P NMR 148.75ppm) to phosphoramidite **6a** (^{31}PNMR: 129.34ppm). [3b]

Phosphoramidite analog **6a** then underwent the coupling reaction with **T_5'OH** to give **7a** and **9a** which was then sulphurized with Beaucage's reagent to give **8a** and **10a** , ^{31}P NMR 68.91 and 69.12ppm. The



diastereomer ratio of **8a** and **10a** was 1:7 when the coupling reaction was performed at RT in acetonitrile. When the coupling reaction was performed at -15°C in deuteriochloroform, the diastereomeric ratio of **8a** and **10a** was 1:70. The product **8a/10a** was purified by chromatography to give only one isomer **10a.** [5b] After hydrolysis of **8a/10a** with 70% TFA at RT, the phosphorothioate dinucleotides **11** and **12** were obtained with Rp : Sp diastereomeric ratio identical to **8a** to **10a**.

It is interesting to note that the different configuration in chiral auxiliary **4** and *ent*-**4** leads to different diastereoselectivity in the coupling reaction and gives the opposite diastereomeric ratio. The chiral auxiliary **4** derived from D-xylose leads to the phosphorothioate dinucleotide **11-Rp**, while the chiral auxiliary *ent*-**4** derived from L-xylose leads to the phosphorothioate dinucleotide **12-Sp**.

In conclusion, we report here that diastereomerically pure cyclic phosphoramidites **6** and **6a** obtained without chromatographic purification lead stereoselectively to Rp and Sp dinucleotide phosphorothioates **11** and **12** respectively.

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3. (a) **6**. m.p. 68-70°C; $[\alpha]^{20}_D$=+62.91° (c=0.5, $CHCl_3$); ^{31}PNMR(202MHz, $CDCl_3$) δ ppm 130.14; ^{1}HNMR(500MHz,$CDCl_3$) δ ppm 8.19 (bs, 1H, NH), 7.48(d, 1H, H-6), 6.34-6.31(dd, 1H, H-1'), 5.90(d, 1H, H-1''), 4.59-4.55(m, 1H, H-3'), 4.43(d, 1H, H-2''), 4.36(m, 1H, H-3''), 4.17 (d, 1H,

H-4"), 4.07 (d, 1H, H-4'), 3.90-3.77 (ABX, 2H, 2xH-5'), 3.47-3.41(m, 2H, H-5", NCH), 3.06-3.01 (m, 1H, H-5"), 2.39-2.35(m, 1H, H-2'), 2.11-2.06(m, 1H, H-2'), 1.90(d, 3H, MeC=C), 1.97-1.62(m, 8H, cyclopentylidene protons), 1.13-1.10(dd, 6H, **Me$_2$**CH), 0.91(s, 9H, t-BuSi), 0.11(d, 6H, Me$_2$Si); HRMS(FAB, glycerol): m/e calcd. for $C_{29}H_{49}N_3O_9PSi$ [MH$^+$]: 642.2975, found 642.2973.

(b) **6a**. m.p. 99 - 101 ^{0}C; $[\alpha]^{20}_D$= -72.00^o (c=0.5, $CHCl_3$); ^{31}P NMR (202MHz, $CDCl_3$) δ ppm 129.34; ^{1}H NMR (500MHz, $CDCl_3$) δ ppm 8.77 (bs, 1H, NH), 7.46(s, 1H, H-6), 6.33-6.30(dd, 1H, H-1'), 5.88(d, 1H, H-1"), 4.56-4.53(m, 1H, H-3'), 4.43(d, 1H, H-2"), 4.35(m, 1H, H-3"), 4.18 (d, 1H, H-4"), 4.05 (m, 1H, H-4'), 3.90-3.76 (ABX, 2H, 2xH-5'), 3.45-3.42(m, 2H, H-5", NCH), 3.03-2.99 (m, 1H, H-5"), 2.38-2.35 (m, 1H, H-2'), 2.12-2.06(m, 1H, H-2'), 1.89(s, 3H, MeC=C),1.96-1.62(m,8H, cyclopentylidene protons), 1.11-1.08 (m, 6H, **Me$_2$**CH), 0.90(s, 9H, t-BuSi), 0.09, (d, 6H, Me$_2$Si); HRMS(FAB, glycerol): m/e calcd. for $C_{29}H_{49}N_3O_9PSi$ [MH$^+$]:642.2975, found 642.2973.

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5. (a) **8**. ^{31}PNMR (202MHz, $CDCl_3$) δ ppm 68.29; ^{1}H NMR (500MHz, $CDCl_3$) δ ppm 7.46(s, 1H, ^{3}H-6), 7.23(s, 1H, ^{5}H-6), 6.37-6.35(dd, 1H, ^{5}H-1'), 6,19-6.17(t, 1H, ^{3}H-1'), 5.87, 5.86(d, 1H, H-1"), 5.15-5.12(dd, 1H, ^{5}H-3'), 4.86-4.83(dd, 1H, H-3"), 4.59, 4.58(d, 1H, H-2"), 4.36-4.33((m, 1H, H-4"), 4.26-4.20(m, 4H, ^{3}H-3', 2x^{3}H-5', ^{5}H-4'), 4.01-4.00(m, 1H, ^{3}H-4'), 3.86(m, 2H, 2x^{5}H-5'), 2.83-2.82(d, 2H, 2xH-5"), 2.79-2.75(septet, 1H, NCH), 2.47-2.43(dd, 1H, ^{5}H-2'), 2.30-2.21(m, 2H, 2x^{3}H-2'), 2.19-2.11(m, 1H, ^{5}H-2'), 1.92(s,3H, $^3CH_3$C=C), 1.90(s, 3H, $^5CH_3$C=C), 1.97-1.61(m, 8H, cyclopentylidene protons), 1.04-1.01(t, 6H, Me$_2$CHN), 0.92(s, 9H, ^{3}t-BuSi), 0.87(s, 9H, ^{5}t-BuSi), 0.13(s, 6H, Me$_2$Si), 0.07(s, 6H, Me$_2$Si). HRMS(FAB, CsI): m/e calcd. for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH$^+$]: 1030.4464, found 1030.4460.

(b) **10a**. ^{31}PNMR (202MHz, CDCl3) δ ppm 69.13; ^{1}H NMR (500MHz, $CDCl_3$) δ ppm 7.46(s, 1H, ^{3}H-6), 7.27(s, 1H, ^{5}H-6), 6.34-6.10(m, 2H, ^{5}H-1', ^{3}H-1'), 5.86, 5.87(d, 1H, H-1"), 5.17-5.14(m, 1H, ^{5}H-3'), 4.83-4.81(d, 1H, H-3"), 4.56, 4.55 (d, 1H, H-2"), 4.38(m, 2H, ^{3}H-3', H-4"), 4.25-4.21(m, 3H, 2x^{3}H-5', ^{5}H-4'), 3..98(m, 1H, ^{3}H-4'), 3.91-3.85(m, 2H, 2x^{5}H-5'), 2.85, 2.84(d, 1H, 2xH-5"), 2.81(m, 1H, NCH), 2.52-2.47(dd, 2H, ^{5}H-2'), 2.25-2.23(m, 2H, 2x^{3}H-2'), 2.08-2.02(m, 1H, ^{5}H-2'), 1.91(s,3H, $^5CH_3$C=C), 1.89(s, 3H, $^3CH_3$C=C), 1.92-1.65(m, 8H, cyclopentylidene protons), 1.05, 1.04(d, 6H, Me$_2$CHN), 0.90(s, 9H, ^{3}t-BuSi), 0.86(s, 9H, ^{5}t-BuSi), 0.11(s, 6H, Me$_2$Si), 0.05(s, 6H, Me$_2$Si); HRMS(FAB, CsI): m/e calcd. for $C_{45}H_{77}N_5O_{14}PSSi_2$ [MH$^+$]: 1030.4464, found 1030.4460.

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