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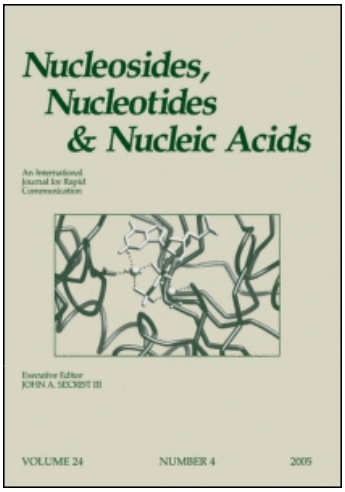

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Imidazo-oxazaphosphorines as Precursors to Chiral Phosphite Triesters

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IMIDAZO-OXAZAPHOSPHORINES AS PRECURSORS TO CHIRAL PHOSPHITE TRIESTERS

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Abstract. New bicyclic imidazo-oxazaphosphorines underwent a highly diastereoselective displacement of the imidazole moiety upon reaction with various alcohols, leading to chiral phosphite triesters as single diastereomers. The introduction of a nucleoside on this bicyclic structure was investigated by several routes, leading to new nucleoside building blocks.

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Phosphorothioate oligonucleotides (S-ODN) have proven, in the past few years, to be interesting candidates for therapeutic use as part of the antisense strategy[1]. They have also led to many other applications, such as the elucidation of the stereochemistry of the mechanism of action of several enzymes[2]. The interest in a stereocontrolled synthesis of S-ODN was prompted by the fact that each time an internucleosidic bridge is made, a pair of diastereomers is generated by virtue of the newly created chirality at the phosphorus center. This led to a number of approaches to control the stereochemistry of S-ODN, the most successful of which has been the oxathiaphospholane method developed by Stec and co-workers[3]. Early in this research, the phosphoramidite approach was left aside because of the problem of epimerization created by the presence of tetrazole, used as the acidic catalyst in the phosphoramidite coupling step. Stec and Zon[4] showed that when a diastereomerically pure phosphoramidite precursor was condensed with the 5'-end of a nucleoside in the presence of tetrazole, a mixture of diastereomers of the resulting dimer was obtained. The mechanism proposed by Stec and Zon, later supported by Berner *et al.*[5], involved an acidic as well as a nucleophilic role of tetrazole.

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Our group tried to address the problem of phosphorus chirality by first synthesizing cyclic chiral precursors in the form of oxazaphosphorinanes[6,7] derived from γ-aminoalcohols. Initial results showed that diastereomerically pure chiral oxazaphosphorinanes bearing a nucleoside as a substituent on the phosphorus atom could indeed be synthesized, thus avoiding a tedious separation of diastereomers. However, the use of tetrazole as a catalyst confirmed Stec's hypothesis and led to high levels of epimerization during the coupling step with a second nucleoside. The replacement of tetrazole by 2-bromo-4,5-dicyanoimidazole led to higher diastereoselectivity, however epimerization was still observed to some extent[6]. Use of a more complex γ-aminoalcohol derived from xylose by Jin *et al.*[7] definitely showed that this method could lead to phosphite triesters in high diastereomeric ratios, after optimization of the coupling conditions. After removal of the chiral auxiliary in acidic conditions, T-T phosphorothioate dimers were thus obtained in diastereomeric ratios in the order of 70:1.

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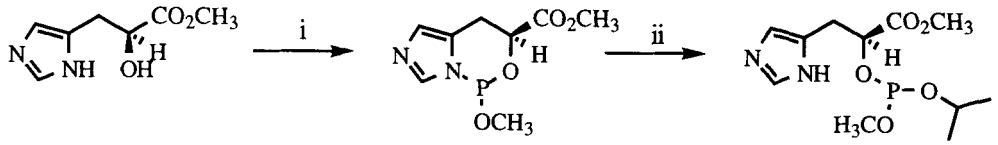
We initially chose another direction to circumvent the problem of epimerization at the phosphorus atom, and tried to replace the amine moiety by an azole, which should not require catalysis by tetrazole. In other words, by creating a more reactive intermediate, the catalyst would be incorporated directly into the precursor. Preliminary results have been published[8], the present article gives a detailed account of our effort in this direction.

Several phosphorotetrazolides[9], phosphorotriazolides[10] and phosphorimidazolides[11] derivatives were reported in the literature. We chose the imidazole group as a suitable candidate, since it would certainly have the highest stability as compared to tetrazole and triazole, and because chiral imidazole derivatives could be obtained from naturally occurring histidine.

Thus, methyl (*S*)-2-hydroxy-3-(imidazol-5-yl)-propionate hydrochloride **1** was synthesized by diazotization of (-)-L-histidine followed by esterification as described by Noordam *et al.*[12]. Chiral precursor **1** gave, upon reaction with methyl dichlorophosphite in dichloromethane in the presence of triethylamine (Scheme 1), a single compound as indicated by a single resonance signal at 143.5 ppm. This signal was attributed to imidazo-oxazaphosphorine **2**, which was too reactive to be purified. Introduction of isopropanol *in situ* led to another single resonance signal at 140.9 ppm, attributed to phosphite triester **3**. These experiments suggested that imidazo-oxazaphosphorine **2** could be formed as a single diastereomer and its reaction with isopropanol led to triester **3** as a single diastereomer, with no need of an external



1 2 3

δ 143.5 δ 140.9

i. PCl_2OCH_3, CH_2Cl_2, Et_3N, 0°C; ii. iPrOH *in situ*

Scheme 1

catalyst such as tetrazole. However, it remained to be proven experimentally with a simpler system.

Chiral (*S*)-1-(imidazol-2-yl)-propan-2-ol **5**, obtained in 75% yield from N-tritylimidazole in two steps following a method developed by Kirk[13], was then investigated as a possible chiral auxiliary (scheme 2). Upon reaction with methyl dichlorophosphite, it gave initially two diastereomers of **6** as indicated by ^{31}P NMR signals at 120.6 (minor) and 118.8 ppm (major). After 20-30 min. standing at RT, the minor isomer disappeared and only one product remained at 118.8 ppm. The same behavior was observed when **5** was reacted with ethyl dichlorophosphite (120.4 and 118.3 ppm), eventually leading to a single compound **7** appearing at 118.3 ppm. Upon introduction of an alcohol *in situ*, a single resonance signal was observed, indicating that the ring opening to yield phosphite triesters **8**, **9** and **10** was highly diastereoselective (Scheme 2). Subsequent sulfurization led to the corresponding phosphorothioate triesters **11**, **12** and **13** as single diastereomers[8], which could then be purified and characterized. When the addition of alcohol was done before the equilibration of **7** to a single diastereomer, two diastereomers of phosphite triester **9** were observed at 141.1 and 140.6 ppm. This proved that the single signals observed for triesters **9** and **12** were not the result of superposition of diastereomeric NMR signals, but actually corresponded to a highly diastereoselective ring opening to give triester **9**, confirming the observations done on imidazo-oxazaphosphorine **2**. Essentially, this series of experiments constituted a new approach to the highly diastereoselective synthesis of phosphite triesters and their corresponding phosphorothioate triesters.

In order to apply this new method to the synthesis of a phosphorothioate dimer, two requirements had to be met. The first one was to replace the ethyl group on bicyclic **7**

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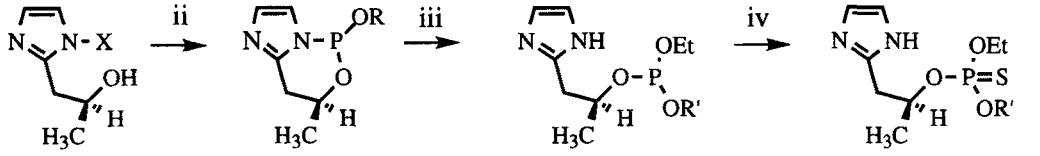

4 X = Tr
5 X = H
i

6 (R=Me)
7 (R=Et)

8 (R'=Bn)
9 (R'=iPr)
10 (R'= 5'tBDMS-T)

11 (R'=Bn)
12 (R'=iPr)
13 (R'= 5'tBDMS-T)

i. 5% AcOH, MeOH Rfx;
ii. PCl_2OR, $5Et_3N$, CH_2Cl_2, 0°C to RT;
ii. R'OH *in situ*; iv. S_8 *in situ*

Scheme 2

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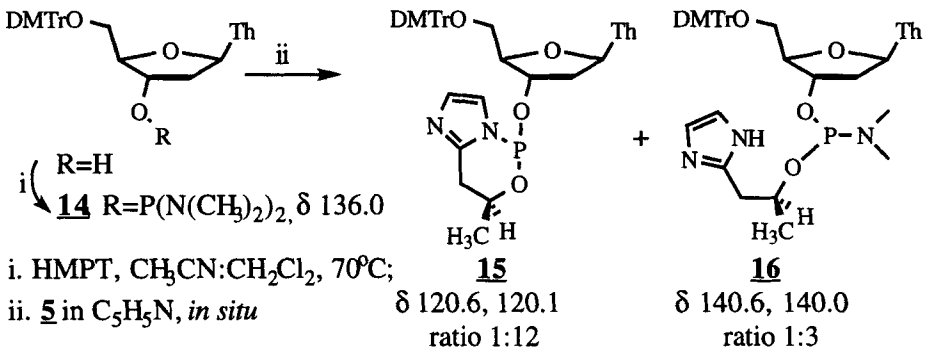
by a 5'-protected nucleoside, the second to modify chiral auxiliary **5** in such way as to allow for its removal at the end of the sequence. We present our effort to address the first requirement in the following section.

We initially expected the direct reaction of imidazolylpropanol **5** with phosphorus trichloride to yield the corresponding chloro substituted imidazo-oxazaphosphorine. However, this intermediate could not be formed under a variety of conditions (variations of the solvent, base and temperature). Consequently, the nucleoside moiety had to be introduced by first being activated at the 3'-position, then condensed with auxiliary **5**.

The direct reaction of 5'-O-tBDMS-thymidine with phosphorus trichloride at low temperature in the presence of a base gave a mixture of the corresponding alkyl dichlorophosphite, dialkyl chlorophosphite and trialkyl phosphite in various ratios depending upon the reaction conditions. Similar results were obtained when the 3-NH of thymidine was methylated.

Dimethylamine was subsequently chosen as a potential leaving group (Scheme 3). The direct reaction of 5'-O-DMTr-thymidine with HMPT in a 2:1 mixture of acetonitrile and dichloromethane led to phosphorodiamidite **14** (δ 136.0 ppm by ^{31}P NMR) in 85% yield, as evaluated by ^{31}P and ^{1}H NMR. The same compound could be obtained by reaction of 5'-O-DMTr-thymidine with HMPT in the presence of tetrazole. In both cases, chromatographic purification led to a large extent to decomposition of the product. Reaction of the crude product obtained by the first method with **5** in acetonitrile at 65°C led to two sets of products. The first set, accounting for 70% of the



Scheme 3

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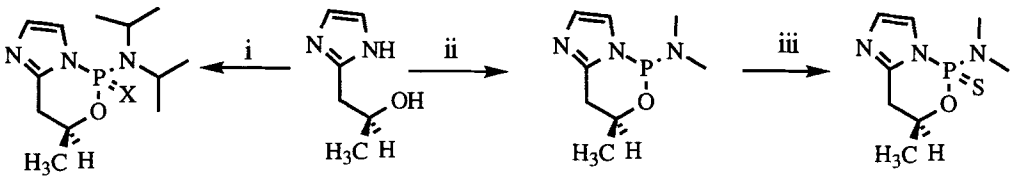
mixture, was attributed to both diastereomers of phosphoramidite derivative **16**, having resonance signals at 140.6 and 140.1 ppm in a 1:3 ratio. The second set, accounting for 30% of the mixture, was attributed to two diastereomers of imidazo-oxazaphosphorine **15** in a ratio of 1:12 given that their chemical shift was very similar to those of **6** and **7**. It seemed that the imidazole group was not nucleophilic enough to displace the dimethylamine group. To verify this assumption, HMPT was reacted directly with **5** (scheme 4). It required 20h heating at 65°C to observe complete disappearance of the signal corresponding to HMPT, resulting in two products **17** having resonance signals at 127.4 and 103.8 ppm, in a ratio of 1:2. Sulfurization in the presence of Beaucage's reagent[14] led to the corresponding thio diastereomers **18**, appearing at 81.8 and 62.4 ppm in the same ratio. However, these products decomposed upon purification. The structures were attributed by comparison to the products of the direct reaction of **5** with diisopropylphosphoramidous dichloride, which appeared as a pair of diastereomers **19** at 126.0 and 103.8 ppm, and which upon sulfurization gave diastereomers **20**. Their structure was confirmed after purification by ^{1}H, ^{13}C, ^{31}P NMR and MS.

As an alternative strategy, tri(imidazol-1-yl) phosphine **21** was considered as a potential precursor. The synthesis of this very reactive species, reported by Shimidzu *et al.*[15] as a good phosphorylating reagent able to react with a diol in the presence of an alcohol, was first re-investigated. Instead of using the method described by Shimidzu *et al*, involving the direct reaction of 6 eq. imidazole with phosphorus trichloride in THF leading to a crude solution of **21**, 1-trimethylsilylimidazole was reacted with phosphorus trichloride in benzene. This resulted in the formation of the desired



19(X=lone pair) δ 126.0; 103.8 (2:1)
20(X=S) d 58.8: 59.1 (2:1)
5
17 δ 127.4; 103.8 (1:2)
18 δ 81.8; 62.4 (2:1)

i. iPr_2NPCl_2, Et_3N, CH_2Cl_2; ii. HMPT, CH_3CN, 65°C, 20h; iii. Beaucage's reagent

Scheme 4

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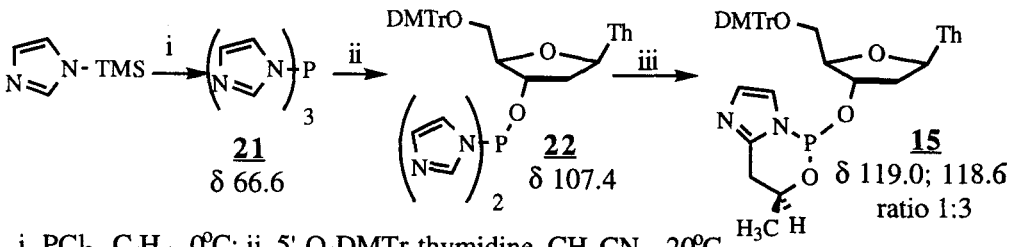
phosphine and trimethylsilyl chloride, the latter being removed with the solvent by evaporation. Phosphine **21** was thus obtained as a pure white pyrophoric solid. With this pure compound, different solvents could be used to react with an alcohol. Our goal was to condense this reagent with 5'-O-DMTr-thymidine in order to form the activated nucleoside **22**. It turned out that, whereas a double displacement of tri(imidazol-1-yl) phosphine **21** by a diol was an entropically favored process[16], a single displacement was difficult to perform chemoselectively. When 5'-O-DMTr-thymidine was reacted with **21** in different solvents (dichloromethane, acetonitrile, pyridine, THF) and at various temperatures (-78, -40, -20, 0°C or RT), a mixture of monosubstitution, disubstitution and trisubstitution products was obtained. The best results were obtained in acetonitrile at -20°C. The amount of **22** thus obtained was 70%, plus some disubstitution product and starting **21**. To this mixture was added a solution of **5** in pyridine at -20°C (Scheme 5). The resulting imidazo-oxazaphosphorine **15** was obtained as a mixture of diastereomers in a ratio of only 1:3, their ^{31}P NMR signals being 119.0 and 118.6 ppm respectively. This ratio did not change upon heating, in contrast with the case when an exocyclic ethyl substituent was present or when dimethylamine was used as a leaving group. We then decided to synthesize an activated nucleoside precursor to **15** that would be isolable.

Helinski *et al.*[17] recently reported the use of 4-nitrophenoxy group as a good leaving group in basic conditions on trivalent phosphorus derivatives, allowing them to synthesize methylphosphonate and phosphorothioate dimers efficiently. We therefore considered another type of activation involving the synthesis of nucleosides functionalized with substituted phenol derivatives at the phosphorus atom.



i. PCl_3, C_6H_6, 0°C; ii. 5'-O-DMTr-thymidine, CH_3CN, -20°C
iii. **5** *in situ*

Scheme 5

Towards this end, N,N-diisopropyl bis-O-aryl phosphoramidite derivatives **23**, **24**, **25**, **26** were synthesized from N,N-diisopropyl phosphoramidous dichloride and the corresponding sodium phenoxide salt (Scheme 6). They all could be purified by flash chromatography and characterized. Among these, only 4-chloro substituted phosphoramidite **26** reacted with isopropanol in the presence of tetrazole to give triester **27**. The more electron defficient phosphoramidites **23**, **24**, **25** did not undergo the same acid-catalyzed displacement, even in the presence of the stronger acid 2-bromo-4,5-dicyanoimidazole. Only a trace of triester was observed in the case of phosphoramidite **24**.

On the other hand, the reaction of **5** with these phosphoramidite derivatives in the presence of 2.2 eq. DBU gave the opposite behavior. Phosphoramidite **26** did not undergo displacement under these conditions, whereas **23**, **24** and **25** gave, after 30 min., two diastereomers of the desired imidazo-oxazaphosphorine **19** in a ratio of 2:1, appearing at 126.0 and 103.8 ppm respectively. These two diastereomers were then sulfurized in the presence of Beaucage's reagent to give the corresponding thio analogues in the same ratio (58.8 and 51.9 ppm by ^{31}P NMR). The 4-chloro substituted phenoxide was not a good enough leaving group to be displaced by imidazolylpropanol **5** in the presence of DBU. In contrast, the 4-nitro, 2,4-dichloro and 2,6-dichloro phenoxides appeared to be good leaving groups on **23**, **24** and **25**. However the nucleoside could not be introduced by acid-catalyzed displacement of the diisopropylamino group of **23**, **24** or **25** since the latter was no longer basic enough for such reaction.

Consequently, instead of using phosphoramidite derivatives, we tried to use directly tri(O-aryl) phosphite triesters. Tri(2,6-dichlorophenyl) phosphite **28** was synthesized

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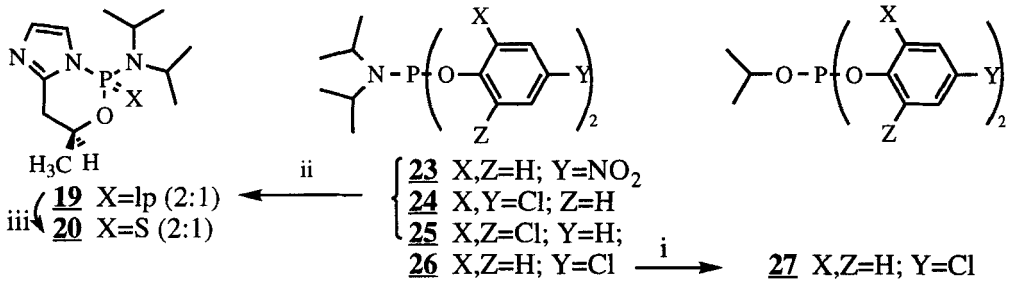

i. iPrOH, tetrazole, THF or CH_3CN; ii. **5**, 2.2 DBU, THF; iii. Beaucage's reagent

Scheme 6

from the corresponding sodium phenoxide salt and phosphorus trichloride and was purified by flash chromatography (141.7 ppm by ^{31}P NMR). The analogous tri(4-nitrophenyl) phosphite (125.7 ppm) and tri(2,4-dichlorophenyl) phosphite (129.0 ppm) decomposed during chromatographic purification.

Subsequently, tri(2,6-dichlorophenyl) phosphite **28** was used as an activated phosphite triester to functionalize the 3'-end of 5'-O-tBDMS-thymidine (Scheme 7). Upon reaction of **28** with 5'-O-tBDMS-thymidine in the presence of 1.1 eq. DBU in dry THF, the desired activated phosphite triester **29** was obtained and could be purified by flash chromatography in 65% yield (^{31}P NMR signal at 131.3 ppm). Activated triester **29** was then reacted with imidazolylpropanol **5**, giving two sets of products in a ratio of 1.2:1. The first set was composed of both diastereomers of imidazo-oxazaphosphorine **30** in a ratio of 12:1 (121.9 and 122.5 ppm respectively), the second set was phosphite triester **31** (140.0 ppm). Sulfurization of the reaction mixture in the presence of Beaucage's reagent led to both diastereomers of thio-analogue **32**, appearing at 53.0 and 53.3 ppm in a ratio of 12:1, as well as the phosphorothioate triester derived from **31**. Only the major diastereomer of **32** could be isolated as a pure product from this reaction mixture and it could thus be characterized. There again, the newly formed imidazo-oxazaphosphorine **30** could be observed but was too reactive to be obtained as a pure product in the reaction conditions used.

In conclusion, imidazo-oxazaphosphorines turned out to be good candidates for the stereocontrolled synthesis of a few simple phosphite triesters such as **3**, **8**, **9** and **10** as well as phosphorothioate triesters **11**, **12**, **13**, establishing a new approach to the synthesis of chiral phosphite triesters. So far, all attempts to incorporate cleanly a

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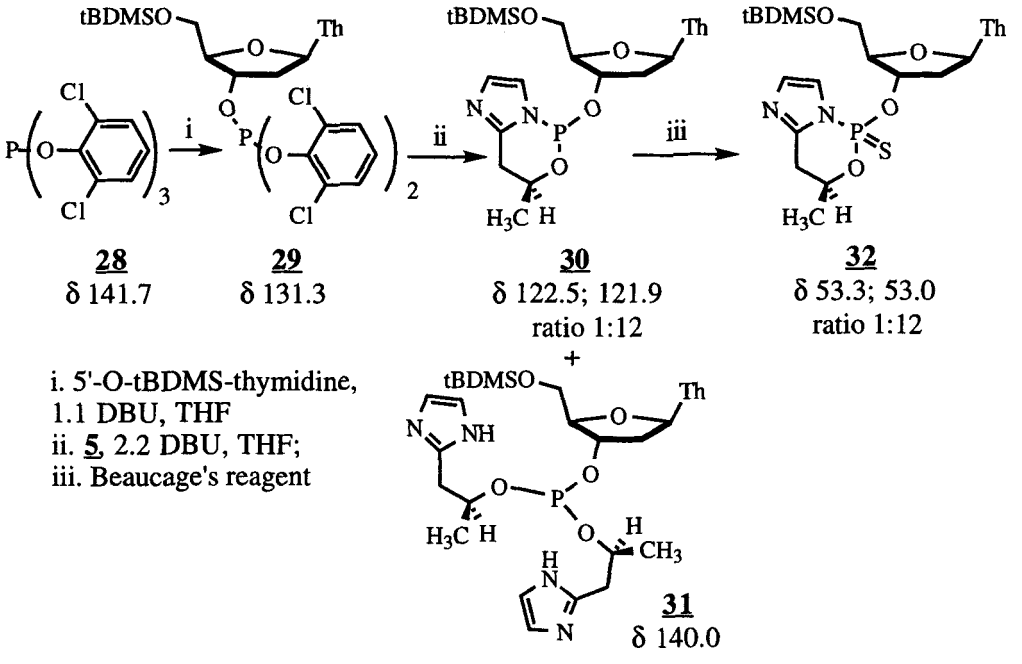

Scheme 7

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nucleoside as the exocyclic substituent on the phosphorus atom failed, mostly due to the high reactivity of imidazo-oxazaphosphorines. However, this study led to the elaboration of useful building blocks for the functionalization of nucleosides at the 3'-position, such as phosphorodiamidite **14** and activated phosphite triester **29**. It also led to a new synthesis of tri(imidazol-1-yl) phosphine **21** as a pure solid, as well as several imidazo-oxazaphosphorines **17**, **18**, **19**, **20** functionalized with different groups at the phosphorus atom.

Experimental section

General methods

Melting points were obtained on a Gallenkamp MF-370 and are uncorrected. Optical rotation measurements were recorded on a Jasco DIP-140 digital polarimeter. Mass spectra were recorded on a MS25RFA mass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian XL200 spectrometer and are referenced with respect to the residual signals of the solvent.^{31}P NMR spectra were obtained on a

Varian XL300 spectrometer and are referenced with respect to external 85% H_3PO_4. THF was distilled from sodium benzophenone ketyl, acetonitrile and triethylamine from calcium hydride, chloroform and methylene chloride from phosphorus pentoxide, methanol from magnesium. Tetrazole and Beaucage's reagent were kindly supplied by Isis Pharmaceuticals.

(7*S*)-7-carboxymethyl-7,8-dihydro-5-methoxy-imidazo[4,3e]-oxazaphosphorine 2

Methyl (*S*)-2-hydroxy-3-(imidazol-5-yl)propionate hydrochloride **1** (0.236 g, 1.39 mmol) was suspended in 5 ml dry methylene chloride and triethylamine (0.30 ml, 4.59 mmol). The suspension was stirred under Ar at 0°C. Methyl dichlorophosphite (0.15 ml, 1.58 mmol) was then syringed into the mixture. After 16h at RT, it showed a single signal at 143.5 ppm. Isopropanol (122 µl, 1.60 mmol) was then injected into the reaction mixture. After 30 min, the conversion to a single product having a resonance signal at 140.9 ppm was observed. This signal was attributed to phosphite triester **3**. However, the compound decomposed on silica gel duing its purification.

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(*S*)-1-((1-triphenylmethyl)-imidazol-2-yl)-propan-2-ol 4

To a solution of N-tritylimidazole (1.55 g, 5 mmol) in 50 ml dry THF stirred under Ar at -78°C, was added a 2.5 M solution of n-butyllithium in pentane (2.4 ml, 6 mmol). The deep red solution thus obtained was allowed to warm up to 0°C and stirred for 30 min, then cooled down again to -78°C. (*S*)-propylene oxide (0.35 g, 6 mmol) was added dropwise. After 30 min, the solution was allowed to warm up to 0°C and stirred at that temperature for 12h. The solution was poured into 50 ml of a 10% solution of ammonium chloride, and this mixture was extracted with dichloromethane. After flash chromatography (hexane:acetone:triethylamine 78:21:1), 1.44g of the desired product was collected (78 % yield) as a white solid, m.p. 201°C.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40-7.10 (m, 15H), 6.90 (d, 1H, 1.2 Hz), 6.71(d, 1H, 1.2 Hz), 5.83 (b, 1H), 3.40-3.60 (m, 1H), 1.78-2.05 (ddd, 2H, 3.2 Hz, 8.5 Hz, 16.2 Hz), 0.81 (d, 3H, 6.0 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 149.2, 142.1, 129.6, 127.9, 127.7, 124.7, 121.0, 74.6, 65.0, 38.1, 22.3; MS (CI) m/z 369 (M+H); $[\alpha]_D^{295}$ -17.9° (c 0.85, $CHCl_3$).

(*S*)-1-(imidazol-2-yl)-propan-2-ol 5

A solution of N-tritylimidazolylpropanol **4** (2.39 g, 6.51 mmol) in 80 ml methanol containing 4.3 ml glacial acetic acid was refluxed for 12h. The mixture was

concentrated *in vacuo* and 50 ml of cold water was added to it. The mixture was cooled down to 0°C then filtered, the precipitate was washed with cold water (10 ml). The filtrate was evaporated twice *in vacuo*, then the residual yellow oil was redissolved in 50 ml dry methanol and passed through the weakly basic anion exchange resin (hydroxide form) IRA-68. The solution was then evaporated *in vacuo* to yield a solid residue that was recrystallized from methanol:ethyl acetate to 0.76 g (93.5%) of a white solid, m.p. 119-121°C.

^{1}H NMR (200 MHz, CD_3OD) δ 6.96 (s, 2H); 3.96 (m, 1H); 2.4-2.65 (ddd, 2H, 6.3 Hz, 6.7 Hz, 14.5 Hz); 0.87 (d, 3H, 6.3 Hz); ^{13}C NMR (67.9 MHz, CD_3OD) δ 145.8; 121.2; 67.1; 38.4; 23.1; MS (CI) m/z 127 (M+H); $[\alpha]_D^{295}$ +13.4° (c 0.50, CH_3OH).

(*7S*) 7,8-dihydro-5-alkoxy-7-methyl-imidazo[3,4-a]oxazaphosphorine 6 (R=Me) and 7 (R=Et)

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In a dry NMR tube purged with Ar, was introduced (*S*)-(imidazol-2-yl)-propan-2-ol **5** (23.0 mg, 0.20 mmol), then 0.7 ml dry $CDCl_3$, followed by triethylamine (127 µl, 1.0 mmol). The suspension was cooled down to 0°C. Methyl dichlorophosphite (19 µl, 0.20 mmol) or ethyl dichlorophosphite (23 µl, 0.20 mmol) was then introduced, giving an exothermic reaction. Immediately, two products were observed by ^{31}P NMR at 120.6 and 118.8 ppm (R=Me), at 120.4 and 118.3 ppm (R=Et). After 20 to 30 min., only one was present at 118.8 ppm (R=Me) or 118.3 ppm (R=Et). The compounds were too unstable to be isolated.

7: ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 127.57 (d, 5.2 Hz); 115.07 (d, 18.1 Hz); 65.94 (d, 6.3 Hz); 60.23 (d, 19.2 Hz); 32.74 (b, J<0.3 Hz); 20.38 (d, 4.1 Hz); 15.53 (d, 5.0 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 118.3.

Phosphorothioate triesters 11, 12, 13

After obtention of a single signal at 118.3 ppm for **7** as indicated above, the desired alcohol (0.45 mmol) was introduced into the mixture and the tube was shaken. After 20 min, ^{31}P NMR indicated the presence of a single peak at 139.8 ppm **8**, 140.6 ppm **9** or 141.2 ppm **10**. Elemental sulfur (6 mg, 0.2 mmol) was introduced, which immediately produced a single compound having a resonance signal at 66.3 ppm (**11**), 64.8 ppm (**12**) or 66.3 ppm (**13**). The product was then concentrated *in vacuo* and purified by flash chromatography (ethyl acetate/hexanes /triethylamine 79/20/1), to yield 80-90% of pure triester **11**, **12** or **13** as a colorless glass.

11: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35 (s, 5H); 6.81 (s, 2H); 5.00-4.80 (m, 3H);

4.03 (m, 2H); 3.00 (m, 2H); 1.29-1.20 (m, 6H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 143.56; 137.52, 131.23, 130.95, 129.28; 121.35; 81.87 (d, 6.2 Hz); 75.36 (d, 5.8 Hz); 74.10 (d, 6.0 Hz); 36.27; 20.87; 15.63; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 66.3; MS (CI) m/z 341 (M+H).

12: ^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (s, 2H); 4.83-4.97 (m, 1H); 4.65 (dh, 1H, 6.2 Hz, 9.6 Hz,); 4.05 (m, 2H); 2.98-3.20 (dddd, 2H, 15.5 Hz, 6.3 Hz, 4.6 Hz, 1.5 Hz,); 1.27 (m, 12H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 144.00; 121.70; 74.90 (d, 6.1 Hz); 73.80 (d, 5.7 Hz); 64.25 (d, 5.8 Hz); 35.95; 23.40; 21.06; 15.83; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 64.8 ppm; MS (CI) m/z 293 (M+H).

13: ^1H NMR (200 MHz, $CDCl_3$) δ 9.80 (b, 2H); 7.48 (d, 1H, 1.3 Hz); 6.94 (s, 2H); 6.25 (dd, 1H, 9.2 Hz, 5.2 Hz); 4.92 (m, 2H); 4.19 (m); 4.05 (dq, 2H, 9.4 Hz, 7.1 Hz); 3.84 (ddd, 2H, 11.5 Hz, 2.5 Hz, 2.4 Hz); 3.03 (dd, 2H, 5.8 Hz, 1.1 Hz); 2.33 (dd, 1H, 13.3 Hz, 5.2 Hz); 2.03 (m, 1H); 1.88 (d, 3H, 1.3 Hz); 1.39 (d, 3H, 6.2 Hz); 1.3 (dt, 3H, 7.1 Hz, 0.9 Hz); 0.91 (s, 9H); 0.12 (s, 6H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 164.10; 151.07; 144.18; 144.15; 135.33; 111.54; 86.15 (d, 6.6 Hz); 85.24; 79.93 (d, 4.3 Hz); 76.34 (d, 5.7 Hz); 65.02 (d, 5.6 Hz); 63.82; 39.39 (d, 4.3 Hz); 36.58 (d, 7.5 Hz); 26.09; 21.32; 18.60; 16.05 (d, 6.6 Hz); 12.66; -5.40, -5.50; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 66.3; MS (FAB, NBA) m/z 589 (M+H).

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Bis(dimethylamino)-(5'-(4,4'-dimethoxy)trityl-thymid-3'-yl)phosphorodiamidite 14

In a scrupulously dry glassware, to a solution of freshly distilled HMPT (36 µl, 0.2 mmol) in 5 ml dry THF stirred at 70°C and containing an Ar inlet directly into the solution, a solution of 5'-O-DMTR-thymidine (109 mg, 0.2 mmol) in 1 ml dry dichloromethane was added dropwise over 1h. After 30 min., the ^{31}P NMR signal corresponding to HMPT at 122.7 ppm completely disappeared and gave rise to a signal corresponding to the desired phosphorodiamidite at 136.0 ppm. The mixture was allowed to cool down to ambient temperature and was evaporated *in vacuo*. Upon attempts to purify this compound, most of it decomposed. However, a reasonably pure sample was obtained directly after the solvents were evaporated.

^1H NMR (200 MHz, $CDCl_3$) δ 8.12 (b, 1H); 7.56 (d, 1H, 1.2 Hz); 7.42-6.80 (m, 13H); 6.34 (dd, 1H, 5.7 Hz, 8.4 Hz); 4.42 (m, 1H); 4.10 (m, 1H); 3.88 (dd, 1H, 11.4 Hz, 2.0 Hz); 3.74 (dd, 1H, 11.4 Hz, 3.4 Hz); 3.81 (s, 6H); 2.54, 2.51 (2d, 12H, 5.7 Hz); 2.54-2.50 (m, 1H); 1.98 (m, 1H); 1.88 (d, 3H, 1.2 Hz); 0.90 (s, 9H); 0.09 (s, 6H); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 136.0.

(7S)-5-N,N-dimethylamino-7-methyl-5-thio-imidazo[3,4-a]oxazaphosphorine 18

To a solution of freshly distilled HMPT (182 µl, 1.0 mmol) in 10 ml dry acetontrile stirred at 65°C under Ar, with an Ar inlet into the solution, was introduced a solution of imidazolylpropanol **5** (126 mg, 1.0 mmol) in 2 ml dry pyridine. After 30 min, ^{31}P NMR indicated essentially the presence of HMPT. After 20h only had this signal completely disappeared, giving rise to two signals at 127.4 and 103.8 ppm respectively in a ratio of 1:2, attributed to both diastereomers of **17**. Several hydrolysis products were also observed, accounting for about 30% of the total amount of the mixture. Beaucage's reagent (200 mg, 1.0 mmol) was then introduced, giving 2 signals at 81.8 and 62.4 ppm in a ratio of 2:1 corresponding to **18**, as well as about 30% of products between 0 and 55 ppm. Products decomposed during the purification .

(7*S*)-7,8-dihydro-5-(N,N-diisopropylamino)-7-methyl-5-thio-imidazo [3,4-a]oxazaphosphorine 20

1. From diisopropylphosphoramidous dichloride:

To a solution of dry triethylamine (210 µl, 1.5 mmol) in deuteriochloroform at 0°C, was added N,N-diisopropylphosphoramidous dichloride (42 µl, 0.3 mmol). The mixture was stirred under Ar, and after 15 min. ^{31}P NMR indicated the presence of two signals at 126.0 and 103.8 ppm in a ratio of 2:1 respectively, corresponding to **19**. To the mixture was then added elemental sulfur (11 mg, 0.33 mmol). Complete sulfurization required 3h, giving two compounds **20** having resonance frequencies at 58.8 and 51.9 ppm in a ratio of 2:1. Flash chromatography (ethyl acetate:hexanes:triethylamine 80:10:10) gave the fast-eluting diastereomer in 50.0% yield (43 mg), and then a mixture of the two diastereomers was obtained in 46.2% yield (40.0 mg).

2. From bis-aryl N,N-diisopropyl phosphoramidites 23, 24 or 25

In a dry NMR tube under Ar, into a solution of (*S*)-1-(imidazol-1-yl)propan-2-ol **5** (38 mg, 0.3 mmol) and bis-aryl N,N-diisopropylphosphoramidite (122 mg of **23**, 136 mg of **24** or **25**, 0.3 mmol) in 0.5 ml dry THF was syringed DBU (100 µl, 0.66 mmol. After shaking, ^{31}P NMR showed two signals at 125.9 and 103.6 ppm in a ratio of 2:1. The mixture was sulfurized as previously indicated, and was purified chromatographically.

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Fast-eluting isomer

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (d, 1H, 1.7 Hz); 7.00 (d, 1H, 1.7 Hz); 4.99 (dddq, 1H, 11.7 Hz, 2.7 Hz, 6.8 Hz, 2.4 Hz); 3.64 (dh, 1H, 6.8, 21.7 Hz); 3.10 (m, 1H); 2.90 (dd, 1H, 16.8 Hz, 11.7 Hz); 1.51 (d, 3H, 6.8 Hz); 1.29 (d, 6H, 6.8 Hz); 1.27 (d, 6H, 6.8 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 147.15 (s); 129.75 (d, 13.3 Hz); 117.12 (d, 8.2 Hz); 72.48 (d, 6.0 Hz); 48.17 (d, 5.5 Hz); 33.78 (s); 22.28 (d, 1.4 Hz); 22.24 (s); 21.72 (d, 11.4 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 58.8; MS (EI) m/z 287 (51.6%, $M^{+\bullet}$).

Slow-eluting isomer

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.01 (d, 1H, 1.5 Hz); 6.91 (d, 1H, 1.5 Hz); 4.79 (m, 1H); 3.42 (dh, 1H, 6.7 Hz, 15.1 Hz); 3.03 (ddd, 1H, 16.8 Hz, 2.7; 1.5 Hz); 2.96 (ddd, 1H, 16.8 Hz, 5.9 Hz, 1.2 Hz); 1.44 (dd, 3H, 5.9 Hz, 1.5 Hz); 1.21 (d, 6H, 6.6 Hz); 1.19 (d, 6H, 6.8 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 51.9.

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Tri(imidazol-1-yl) phosphine 21

To a solution of 1-trimethylsilylimidazole (1.32 ml, 9.0 mmol) in 25 ml dry benzene stirred at 0°C under Ar, was introduced phosphorus trichloride (262 µl, 3.0 mmol). After 30 min., the solvent and trimethylsilyl chloride were removed *in vacuo*. Phosphine **21** was collected as a pure white pyrophoric solid.

^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70; 7.20; 7.05; ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 139.67; 133.48; 119.32; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 66.6; MS (EI) m/z 232 (0.5%, $M^{+\bullet}$); 68 (100%, ($C_3H_4N_2$)).

Reaction of 21 with 5'-O-DMTr-thymidine

In a glove box, **21** was weighed (46 mg, 0.2 mmol) and introduced into a dry flask. The flask was sealed with a septum, then the solvent (anhydrous) was introduced. It was cooled down to the appropriate temperature, then a solution of 5'-O-DMTr-thymidine (103 mg, 0.19 mmol) was introduced. ^{31}P NMR showed the appearance of three signals: **21** at 66.6 ppm, di(imidazol-1-yl) (5'-O-dimethoxytritylthymid-3'-yl) phosphine **22** at 107.4 ppm and imidazol-1-yl bis(5'-O-dimethoxytritylthymid-3'-yl) phosphine at 125.4 ppm.

Bis(aryl) (N,N-diisopropyl) phosphoramidites 23, 24, 25, 26

To a suspension of the desired sodium phenoxide salt (3.0 mmol, 452 mg of sodium 4-chlorophenoxide, 483 mg of sodium 4-nitrophenoxide, 555 mg of sodium 2,6-

dichlorophenoxide or 555 mg of sodium 2,4-dichlorophenoxide) in 10 ml dry THF stirred under Ar at 0°C was added N,N-diisopropylphosphoramidous dichloride (464 µl, 3.3 mmol). After 30 min., ^{31}P NMR indicated the presence of a single signals at 144.8, 145.3, 153.6 and 147.2 ppm, corresponding respectively to **26**, **23**, **25** and **24**.

The mixture was then percolated through a 5 cm pad of silica gel and washed with 50 ml dry THF. Evaporation of the solvent *in vacuo* yielded either a white sticky solid or a colorless oily compound that solidified upon standing.

23: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.14 (d, 4H, 9.1 Hz); 7.10 (dd, 4H, 9.1 Hz, 1.7 Hz); 3.70 (dh, 2H, 6.7 Hz, 11.6 Hz); 1.23 (d, 12H, 6.7 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 159.50; 143.10; 125.86; 119.59 (d, 9.3 Hz); 44.70 (d, 12.9 Hz); 24.53; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 144.5; MS (CI) m/z 408 (M+H).

24: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45 (s, 2H); 7.27-6.90 (m, 4H); 3.90 (dh, 2H, 6.9 Hz, 11.1 Hz); 1.25 (d, 12H, 6.9 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 151.0, 149.7, 129.9, 127.5, 120.3, 116.4; 45.3 (d, J=13.7 Hz); 24.1 (d, J=9.5 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 147.2; MS (CI) m/z 456 (M+H).

25: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.22 (m, 4H); 6.87 (m, 2H); 4.12 (m, 2H); 1.31 (d, 12H, 6.5 Hz); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 147.35; 128.81; 128.57 (d, 2.1 Hz); 123.89; 44.83 (d, 15.0 Hz); 24.71 (d, 13.2 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 153.5; MS (CI) m/z 456 (M+H).

26: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.22 (b, 4H); 6.97 (b, 4H); 3.82 (m, 2H); 1.20 (d, 12H, 5.9 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 145.3; MS (CI) m/z 386 (M+H).

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Acid-catalyzed reaction of the phosphoramidites with isopropanol

To a solution of the phosphoramidite (203 mg of **23**, 193 mg of **26**, 228 mg of **24** or 228 mg of **26**, 0.5 mmol) in dry THF (5 ml) stirred under Ar at RT was added a solution of isopropanol (43 µl, 0.55 mmol) and tetrazole (175 mg, 2.5 mmol) in 2 ml dry THF. After 3h, only **26** had reacted completely, giving rise to **27** having a signal at 129.2 ppm. **23** did not show any reaction after 16, nor did **25**. Phosphoramidite **24** showed some trace of a product at 135.0 ppm after 12h.

Tris (2,6-dichlorophenyl) phosphite 28

To a suspension of sodium 2,6-dichlorophenoxide (555mg, 3.0 mmol) in 10 ml dry THF stirred under Ar at 0°C was added freshly distilled phosphorus trichloride (96 µl, 1.1 mmol). After 1h, ^{31}P NMR indicated the presence of a single signal at 141.7 ppm

corresponding to **28**. The solution was then percolated through a 5 cm pad of silica gel and washed with 50 ml dry THF. A white solid was obtained after evaporation.
^{1}H NMR (200 MHz, $CDCl_3$) δ 7.31 (m, 6H); 6.95 (m, 3H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 131.06, 128.54 (b), 128.12 (d, J=12.3 Hz); 125.62 (d, J=7.6 Hz); ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 141.5; MS (EI) m/z 516 ($M^{+\bullet}$).

Bis (2,6-dichlorophenyl) (5'-O-*ter*butyldimethylsilyl)thymid-3'-yl phosphite 29

To a mixture of tris(2,6-dichlorophenyl) phosphite **28** (517 mg, 1.0 mmol) and 5'-O-tBDMS-thymidine (356 mg, 1.0 mmol) in 20 mmol dry THF cooled down to -20°C and stirred under Ar, was added a solution of dry DBU (150 ml, 1.0 mmol) in 5.0 ml dry THF, over 2h. The mixture was then allowed to warm up to RT and was stirred for 30 min. It was purified by flash chromatography using hexanes:ethyl acetate as an eluent (60:40) to a colorless oil, that crystallized upon standing (462 mg, 65 %). m.p. 75-77°C (dec.).
^{1}H NMR (200 MHz, $CDCl_3$) d 8.85 (s, 1H); 7.60 (s, 1H); 7.20-7.40 (m,4H); 6.90-7.00 (m, 2H); 6.45 (dd, 1H, 5.1 Hz; 9.4 Hz); 5.85 (m, 1H); 4.45 (m, 1H); 3.95 (ddd, 2H, 11.4 Hz; 2.0 Hz; 1.5 Hz); 2.65 (dd, 1H, 13.6 Hz; 5.1 Hz); 2.20 (m, 1H); 1.87 (s, 3H); 0.85 (s, 9H); 0.10 (s, 6H); ^{13}C NMR (67.9 MHz, $CDCl_3$) d 163.87; 150.39; 135.43; 129.0, 128.33, 128.12 (d, 14.5 Hz); 125.22 (d, 8.3 Hz); 110.99; 86.89; 84.98; 75.92 (d, 5.2 Hz); 63.34; 40.77; 26.01; 18.37; 12.56; -5.26, -5.36; ^{31}P NMR (109.4 MHz, $CDCl_3$) d 131.3; MS (FAB, NBA) m/z 711 (M+H).

(7*S*)-5-(5'-*ter*butyldimethylsilylthymid-3'-yl)-7,8-dihydro-7-methyl-5-thio-imidazo[3,4-a]oxazaphosphorine 32

In a dry NMR tube were introduced the activated phosphite triester **29** (71 mg, 0.1 mmol) and imidazolylpropanol **5** (13 mg, 0.1 mmol), followed by dry THF (0.5 ml) and DBU (33 µl, 0.22 mmol. After 30 min., ^{31}P NMR showed no further evolution and two sets of peaks were observed: two peaks at 122.5 and 121.9 ppm in a ratio of 1:12, corresponding to imidazo-oxazaphosphorine **30**, as well as triester **31** at 140.0 ppm in a ratio **30**:**31** of 1.2:1. To this mixture was added Beaucage's reagent (22 mg, 0.11 mmol). After 20 min., the signals corresponding to **30** turned into two signals at 53.0 and 53.3 ppm, in a ratio of 12:1. The solvent was removed *in vacuo*, and the products were separated chromatographically, using dry ethyl acetate:triethylamine 85:15 as an eluent. Only the major diastereomer of **32** could be obtained pure in a low yield (25%, 13 mg), as a colorless oil.

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^{1}H NMR (200 MHz, $CDCl_3$) δ 8.16 (b, 1H); 7.47 (b, 1H); 7.12-7.06 (b, 2H); 6.28 (dd, 1H, 4.9 Hz, 9.0 Hz); 5.36 (m, 1H); 4.97 (m, 1H); 4.16 (m, 1H); 3.90 (m, 2H); 3.22 (ddd, 2H, 16.1 Hz, 2.0 Hz, 11.3 Hz); 2.49 (dd, 1H, 13.6 Hz; 4.9 Hz); 2.18 (dd, 1H, 13.6 Hz, 9.0 Hz); 1.90 (s, 3H); 1.60 (d, 3H, 5.9 Hz); 0.90 (s, 9H); 0.15 (s, 6H); ^{13}C NMR (67.9 MHz, $CDCl_3$) δ 164.15; 151.18; 148.21; 135.27; 131.21 (d, 12.5 Hz); 118.24 (d, 6.1 Hz); 114.86; 87.28 (d, 4.2 Hz); 85.95; 76.45 (d, 5.1 Hz); 67.36 (d, 6.1 Hz); 64.23; 41.28 (d, 3.9 Hz); 35.28 (d, 6.9 Hz); 27.12; 23.95; 19.45; 12.54; -5.62, -5.69; ^{31}P NMR (109.4 MHz, $CDCl_3$) δ 53.1; MS (FAB, NBA) m/z 543 (M+H).

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