Indolyl-oxazaphosphorine Precursors for Stereoselective Synthesis of Phosphite Triesters and Dithymidinyl Phosphorothioates

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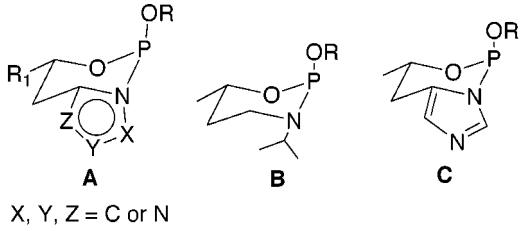
Several novel chiral indolyl-oxazaphosphorines **7** were synthesized, and their potential as precursors to chiral phosphorothioates was evaluated. Reaction of **7** with a thymidine derivative gave phosphite triester **8** with a large degree of stereoselectivity. Sulfurization with Beaucage's reagent provided phosphorothioate triesters **9**. The chiral auxiliary **9b** containing a cyano group could be easily removed with aqueous ammonia to form dithymidinyl phosphorothioate in more than 97% diastereomeric excess. The chiral indolyl-oxazaphosphorines **7** are a new class of precursors for stereoselective synthesis of phosphorothioates.

Introduction

Oligonucleotide phosphorothioates (PS-Oligos) have attracted much attention as a result of 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 is the oxathiaphospholane approach, which has been described by Stec and co-workers.[2] Their chiral precursors, oxathiaphospholanes, were separated chromatographically from a diastereomeric mixture. Recently, we developed a synthesis for chiral cyclic phosphoramidites, which were obtained from xylose and could be used without purification for the preparation of diastereoisomerically enriched dithymidinyl phosphorothioates.[3] This procedure involves an acid-catalyzed coupling step. It turns out that mono- or dimethoxy trityl groups used to protect the 5′-hydroxyl group of nucleosides are not compatible with these acidic catalysts. This encouraged us to look for chiral auxiliaries that might not require the acid-catalyzed coupling step. One way to design such analogue is to incorporate an azole leaving group (see A in Scheme 1) in lieu of the phosphoramidite B.

We have reported on chiral bicyclic imidazo-oxazaphosphorine C, which was obtained with high de from a chiral precursor by a simple equilibration.[4] Imidazo-oxazaphosphorine C coupled with a nucleoside in the presence of triethylamine and gave a phosphite triester

Scheme 1



with excellent de's. However, it was too reactive to be used routinely, and considerable difficulties were encountered in preparing the appropriate starting materials. We therefore investigated different azole groups and evaluated their potential as possible chiral precursors. In our preliminary communications,[5] we have described the usefulness of indole derivative **5a** and **5b** as chiral auxiliaries for stereocontrolled synthesis of phosphite triesters and therefore of phosphorothioates. Here we wish to report the full details of our work in this area.

Results and Discussion

A comparison of imidazole (pK_a 14.10) with the less acidic indole (pK_a 16.97) indicated that the latter may be a suitable leaving group. Thus, indole was reacted with diethyl chlorophosphite in the presence of triethylamine, and stable diethyl indolphosphite **1** (^{31}P NMR, 130.1 ppm) was obtained**,** which could be purified by silica gel column chromatography. The corresponding diethyl benzimidazolphosphite (^{31}P NMR,129.5 ppm) and benzotriazolphosphite (^{31}P NMR, 135.5 ppm) were too unstable for purification and reacted rapidly upon addition of an alcohol in the presence of triethylamine. Interestingly, the displacement of an indole group in **1** by an alcohol required activation by a strong base such as 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), and no reaction occurred in the presence of triethylamine, even after several days. These model reactions suggested that cyclic indolides might be good precursors for the synthesis of chiral phosphorothioates. (*S*)-1-(Indol-2-yl)-propan-2-ol **5a**

(1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543.
(2) (a) Stec, W. J.; Grajkowski, A.; Koziolkiewicz, M.; Uznanski, B. *Nucleic Acids Res.* **1991**, *19*, 5883. (b) Stec, W. J.; Grajkowski, A.; Kobylanska, A.; Karwowski, B.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019. (c) Stec, W. J.; Karwowski, B.; Boczkowska, M.; Guga, P.; Koziolkiewicz, M.; Sochacki, M.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1998**, *120*, 7156.
(3) (a) Xin, Z.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 969. (b) Jin, Y.; Biancotto, G.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 973. (c) Marsault, E.; Just, G. *Tetrahedron* **1997**, *53*, 16945. (d) Yi, J.; Just, G. *J. Org. Chem.* **1998**, *63*, 3647.
(4) Marsault, E.; Just, G. *Tetrahedron Lett.* **1996**, *37*, 977.
(5) (a) Wang, J.-C.; Just, G. *Tetrahedron Lett.* **1997**, *38*, 705. (b) Wang, J.-C.; Just, G. *Tetrahedron Lett.* **1997**, *38*, 3797.

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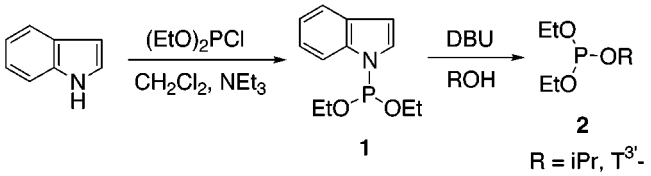
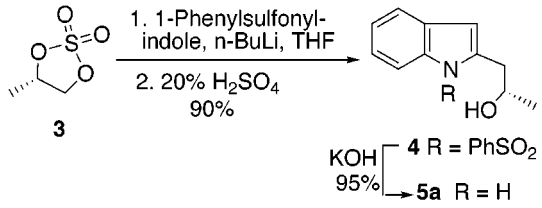
Scheme 2



Scheme 3

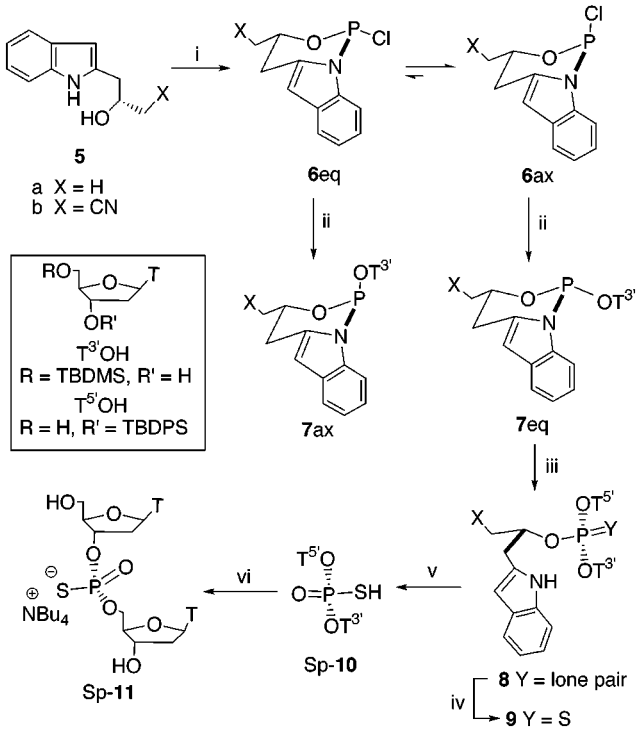


was chosen as a model chiral auxiliary. It was synthesized from the sulfate[6] of (*S*)-propanediol and the anion of 1-phenylsulfonylindole,[7] as outlined in Scheme 3.

Equimolar acetonitrile solutions of **5a** 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 to 60 °C for about 10 h until the ^{31}P NMR showed a major peak at 144 ppm, which indicated the formation of phosphorochloridite **6a**. It probably exists as a rapidly equilibrating mixture of $\mathbf{6a}_{ax}$ and $\mathbf{6a}_{eq}$, in which $\mathbf{6a}_{ax}$ predominates (vide infra). The mixture was cooled to 0 °C, and a solution of 5′-*O*-TBDMS-thymidine ($T^{3'}OH$) in CH_2Cl_2 was added. Two peaks were observed, a major one at 120.67 ppm and a minor one at 121.56 ppm, corresponding to the formation of the two diastereoisomers $\mathbf{7a}_{eq}$ and $\mathbf{7a}_{ax}$. The ratio of the two diastereoisomers of **7a** was moderately affected by the temperature at which $T^{3'}OH$ was added. At room temperature, the ratio was 7:1, and at −78 °C, the ratio increased to 9:1. In our previous studies,[3a,4] the two diastereoisomers at the phosphorus of phosphoramidite B and imidazo-oxazaphosphorine C could be equilibrated by heating their reaction solutions containing triethylammonium chloride to form the thermodynamically more stable form in which the OR group was at the axial position. In marked contrast, $\mathbf{7a}_{ax}$ and $\mathbf{7a}_{eq}$ could not be equilibrated by heating the reaction solution. In addition, no equilibration was observed by heating the $CDCl_3$ solution of **7a** in the presence of triethylamine or aniline hydrochloride. The major isomer $\mathbf{7a}_{eq}$ was hydrolyzed much faster than the minor isomer $\mathbf{7a}_{ax}$. Diastereoisomers $\mathbf{7a}_{ax}$ and $\mathbf{7a}_{eq}$ could not be separated by flash chromatography on silica gel.

The coupling step of **7a** with 3′-*O*-TBDPS-thymidine ($T^{5'}OH$) was carried out in the presence of DBU. The major diastereoisomer $\mathbf{7a}_{eq}$ reacted much faster with $T^{5'}$-OH than the minor axially substituted isomer $\mathbf{7a}_{ax}$. The ^{31}P NMR spectra of the reaction in $CDCl_3$ was monitored at various time intervals (Figure 1). By using 1 equiv of DBU and 1 equiv of $T^{5'}OH$, 95% of $\mathbf{7a}_{eq}$ was converted to phosphite triester **8a** after 5 h at 50 °C, whereas $\mathbf{7a}_{ax}$ almost did not react. After filtration through a short silica gel column to remove DBU, triester **8a** was treated with

Scheme 4[a]



[a] (i) PCl_3, Et_3N, CH_3CN, or THF, 0−60 °C. (ii) $T^{3'}OH$. (iii) $T^{5'}OH$, DBU. (iv) Beaucage's reagent. (v) 28% NH_4OH, 50 °C, 0.5 h. (vi) TBAF, DMF.

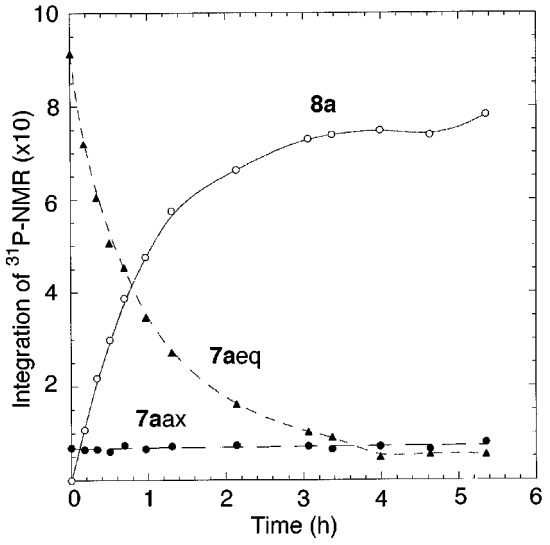


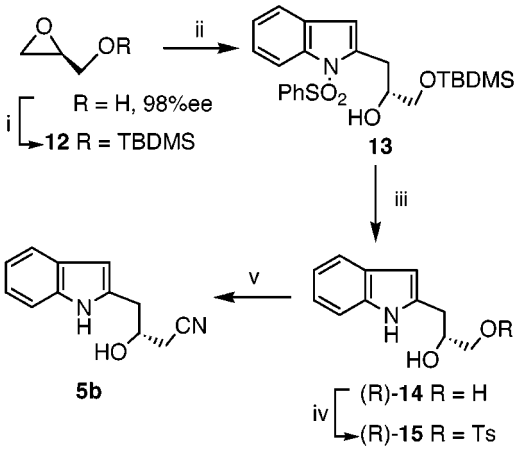
Figure 1. Time course of the coupling reaction of **7a** with $T^{5'}OH$. The reaction was carried out in $CDCl_3$ (1.5 mL) at 50 °C with **7a** (50 mg, 89 μmol), $T^{5'}OH$ (43 mg, 89 μmol), DBU (13 μL, 89 μmol), and 10 μL of trimethyl phosphate as an internal standard.

Beaucage's reagent[8] to give a 73:1 mixture of phosphorothioate triesters **9a**,^{31}P NMR, 66.76 (major) and 66.59 (minor) ppm. The chiral auxiliary **9a** could not be removed with 28% ammonium hydroxide.

Our next step was to develop a chiral auxiliary that could be removed at the end. We considered three different approaches: (a) classical β-elimination of a

(6) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.
(7) Saulnier, M.; Gribble, G. W. *J. Org. Chem.* **1982**, *47*, 757.
(8) Iyer, R. P.; Egan, W.; Regan, J. B.; Beaucage, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 1253.

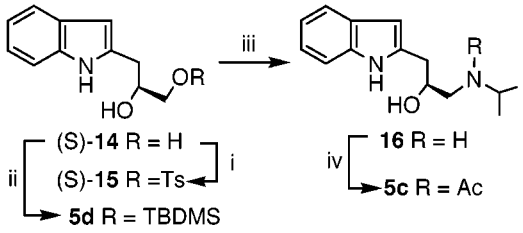
Scheme 5[a]



[a] (i) TBDMSCl, DMAP, Et_3N, CH_2Cl_2, 75%. (ii) 1-Phenylsulfonyl-indole, *n*-BuLi, −78 → 25 °C, overnight, 48%. (iii) KOH, CH_3OH/H_2O (3:1), reflux, 87%. (iv) TsCl (1.1 equiv), pyridine, 0 °C, overnight, 90%. (v) NaCN, LiCN, DMF, 100 °C, 3 h, 74%.

cyanoethyl group (see **5b**, X = CN);[9] (b) neighboring group participation, as applied to nucleosides by Iyer et al. (see **5c**, X = N(^{i}Pr)Ac);[10] and (c) fluoride-catalyzed desilylation resulting in the formation of an epoxide (see **5d**, X = TBDMSO).[11] The reaction of silyl-protected glycidol **12** with the anion of 1-phenylsulfonylindole gave indole derivative **13**. Both protecting groups on **13** were removed with potassium hydroxide to provide diol **14**. The primary hydroxyl group of **14** was selectively tosylated and reacted with cyanide in DMF to give nitrile **5b**. During the last step, partial epimerization on the chiral center occurred,[12] as the ee in **5b** was only 91% as determined with a chiral column (Chiralcel OD). After recrystalization in $CHCl_3$, **5b** was obtained in 96% ee from the recrystallization mother liquor.

Scheme 6[a]



[a] (i) and (ii) see steps iv and i in Scheme 5. (iii) (*S*)-**15**, isopropylamine, 110 °C, overnight, 81%. (iv) Acetic anhydride, CH_2Cl_2, 5 h, 92%.

The acetamide derivative **5c** and silyl-protected indole derivative **5d** were synthesized from diol (*S*)-**14**,[13] as shown in Scheme 6.

Several solvents were tested for the formation of cyclic compound **6b**. In THF at −78 °C, the reaction of cyano derivative **5b** with PCl_3 was complete in several minutes to give phosphorochloridite **6b** with a major peak at 144 ppm, whereas in acetonitrile or dichloromethane the reaction needed several hours. Thymidine derivative T$^{3'}$-OH was then added at −78 °C. After 30 min, the reaction mixture was purified by silica gel chromatography to provide indolyl-oxazaphosphorines **7b**. The ratio of the two diastereomers **7** was affected by workup, especially by flash silica gel chromatography, because $\mathbf{7}_{eq}$ is much less stable to moisture or air. By using preparative TLC, which was developed under argon, two diastereomers, $\mathbf{7b}_{eq}$ and $\mathbf{7b}_{ax}$ were obtained in a ratio of 30:1 as established by ^{31}P NMR [120.78 (major) and 120.65 (minor) ppm].[14]

As for $\mathbf{7a}_{eq}$, equatorial indolyl-oxazaphosphorine $\mathbf{7b}_{eq}$ reacted much faster than the axial ($\mathbf{7b}_{ax}$). To get **8b** with high de, excess starting material **7b** was used; 1.3 equiv of **7b** (in a ratio of 30:1) was treated with 1 equiv of T$^{5'}$-OH to afford phosphite triester **8b**. With 2 or 5 equiv of DBU at room temperature, the coupling was complete in less than 10 or 5 min, respectively. Interestingly, the corresponding reaction of **7a** carrying no cyano group with T$^{5'}$OH required several hours. The explanation may be the electric induction on the phosphorus atom by the strong electron-withdrawing cyano group, which made the phosphorus in **7b** more positive. This may make it easily attacked by an alcohol. Phosphite triester **8b** was stable to DBU under the conditions used. Here the general procedure for the coupling reaction was carried out by first mixing the two solid reagents **7b** and T$^{5'}$OH and then introducing solvent and DBU under argon. No side products were detected. After filtration through a short silica gel column to remove DBU, sulfurization with Beaucage's reagent afforded phosphorothioate triester as a single diastereomer **9b** with a peak at 66.55 ppm in its ^{31}P NMR spectrum.

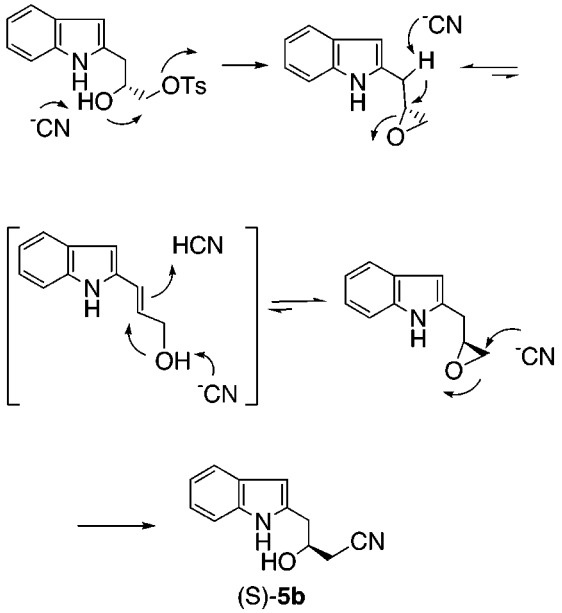
The chiral auxiliary **9b** was easily removed with 28% ammonium hydroxide at 50 °C for 30 min to form Sp-**10** (^{31}P NMR 58.98 ppm). Deprotection of silyl groups with

(9) (a) Sinha, N. D.; Biernat, J.; Koster, H. *Tetrahedron Lett.* **1983**, *24*, 5843. (b) Sinha, N. D.; Biernat, J.; McManus, J.; Koster, H. *Nucleic Acids Res.* **1984**, *12*, 4539.

(10) (a) Iyer, R. P.; Yu, D.; Devlin, T.; Ho, N.-H.; Agrawal, S. *J. Org. Chem.* **1995**, *60*, 5388. (b) Iyer, R. P.; Yu, D.; Ho, N.-H.; Tan, W.; Agrawal, S. *Tetrahedron: Asymmetry* **1995**, *6*, 1051.

(11) (a) Molander, G. A.; Swallow, S. *J. Org. Chem.* **1994**, *59*, 7148. (b) Miyazawa, M.; Ishibashi, N.; Ohnuma, S.; Miyashita, M. *Tetrahedron Lett.* **1997**, *38*, 3419.

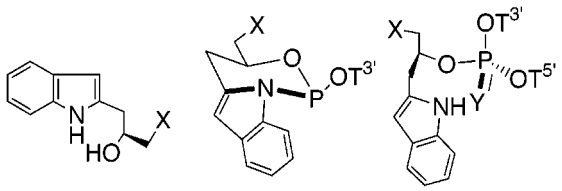
(12) We thank Dr. S. L. Beaucage for proposing an epimerization mechanism.



(13) The synthesis of diol (*R*)- and (*S*)-**14** was scaled up by Dalton Chemical Laboratories, Inc., Ontario, Canada. The chiral auxiliaries **5c** and **5d** were synthesized in (*S*) configuration because of the avaiable of starting material (*S*)-14.

(14) Although nitrile **5b** (96% ee) was used, only two diastereomers of indolyl-oxazaphosphorine **7b** were observed. Probably because of the limitation of detection, the minor compounds could not be detected.

Scheme 7



TBAF afforded dithymidinyl phosphorothioate Sp-**11**. The two diastereomers of Sp- and Rp-**11** can be easily identified by the ^{31}P NMR in D_2O. There is more than 0.3 ppm difference in their chemical shifts, and the Rp-isomer is at lower field. Only one diastereomer Sp-**11** (^{31}P NMR in D_2O 55.45 ppm) was identified from this experiment. In a parallel run, (*S*)-**5b** was transformed to **7Sb** (a mixture of two diastereomers in a ratio of 6:1) after purification by flash column chromatography.[15] Phosphorothioate Rp-**11** was obtained in a ratio of 24:1 by the reaction of equimolar **7Sb** and T$^{5'}$OH followed by sulfurization and desilylation. The absolute stereochemistry of dimers Rp- and Sp-**11** were confirmed by snake venom phosphodiesterase and P1 nuclease digestion and HPLC analysis.[16] The stereoconfiguration of **11** proved that the replacement of the indole group in indolyl-oxazaphosphorine **7** with an alcohol proceeded with inversion.

As described for the formation of **7a**, acetoamide derivative **5c** was transformed to indolyl-oxazaphosphorine **7Sc** with two diastereomers in a ratio of 6.8:1, a major one at 120.68 ppm and a minor one at 120.76 ppm. Phosphorothioate triester **9Sc** (^{31}P NMR in THF, 68.89 ppm) was obtained via the intermediate phosphite triester **8Sc** (^{31}P NMR in THF, 143.26 ppm). In contrast to the cyano derivative **9b**, the acetoamide derivative **9Sc** in tetrahydrofuran solution hydrolyzed spontaneously upon sulfurization within several hours to provide **10** (^{31}P NMR in THF, 58.65 ppm). By adding a base such as triethylamine, the reaction was complete in several minutes.

We also briefly investigated the chiral auxiliary **9Sd** (X = TBDMSO). Using TBAF, after a week at 50 °C, phosphorothioate **11** was released only partially as established by ^{31}P NMR. So far we have not found an efficient way to eliminate the silyloxyl chiral auxiliary.

The cyano indolyl-oxazaphosphorine was tried on the solid-phase synthesis. Unfortunately, its application to solid phase was not very successful, because a large amount of DBU has to be used to activate the nucleoside on solid support.[17] Work on the use of the acetamido group in solid-phase synthesis is at present being investigated and will be reported in due course.

Conclusion

We investigated a number of chiral indolyl-oxazaphosphorines **7** as potential precursors for the stereocontrolled synthesis of phosphorothioates. The indole group in **7** could be diastereoselectively replaced by a nucleoside, and the replacement proceeds by inversion of configuration. The chiral auxiliaries **9b** and **9Sb** containing a cyano group could be easily removed by aqueous ammonia, and the acetamido chiral auxiliary **9Sc** was eliminated spontaneously. Even though the application on solid-support synthesis was not successful, we demonstrated that indole is a good leaving group and can be advantageously modified to potential chiral precursors for stereocontrolled synthesis of phosphite triesters and phosphorothioates.

Experimental Section

Melting points (mp) are uncorrected. NMR spectra were recorded at 500 or 270 MHz for ^{1}H NMR, 125 or 67.9 MHz for ^{13}C NMR, and 202 MHz for ^{31}P NMR. THF was dried by distillation on sodium−benzophenone ketyl, dichloromethane from phosphorus pentoxide, acetonitrile and triethylamine on calcium hydride, and pyridine on barium oxide. DBU was distilled under vacuum and then stored over 4 Å Linde molecular sieves under argon. PCl_3 was first degassed by refluxing for 2 h under argon followed by fractional distillation and was used within a week. Beaucage's reagent and 3′-*O*-TBDPS-thymidine were generously given by ISIS Pharmaceuticals (Carlsbad, CA).

(*S*)-1,2-Propanediol Cyclic Sulfate (3). To a 100-mL, two-necked, round-bottomed flask equipped with a reflux condenser and topped with a $CaCl_2$ drying tube, connected to an HCl trap, and a rubber septum were added (*S*)-1,2-propanediol (2.3 g, 40 mmol) and CCl_4 (20 mL). Thionyl chloride (4 mL, 54.8 mmol) was added via a syringe, and the resulting solution was refluxed for 30 min. The solution was then cooled with an ice bath and diluted with CH_3CN (20 mL). Next, $RuCl_3 \cdot 3H_2O$ (7.8 mg, 0.03 mmol) and $NaIO_4$ (12 g, 56 mmol) were added followed by water (40 mL). The resulting mixture was stirred at room temperature for 1 h. The mixture was then diluted with ethyl acetate, and the two phases were separated. The organic layer was washed with water, saturated aqueous sodium bicarbonate, and brine. After drying over anhydrous sodium sulfate, the solution was filtered through a small pad of silica gel to remove the brown impurities. The filtrate was concentrated to afford 4.0 g (98%) of (*S*)-1,2-propanediol cyclic sulfate **3** as a colorless liquid.^{1}H NMR (270 MHz, $CDCl_3$): δ 5.10 (ddq, J = 8.2, 6.0, 6.2 Hz, 1H), 4.72 (dd, J = 8.7, 6.0 Hz, 1H), 4.28 (dd, J = 8.7, 8.2 Hz, 1H), 1.55 (d, J = 6.2 Hz, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 80.0, 74.3, 17.7.

(*S*)-(1-Phenylsulfonylindol-2-yl)propan-2-ol (4). To a solution of 1-phenylsulfonylindole (2.57 g, 10 mmol) in dry THF (30 mL) was added dropwise 8 mL of *n*-butyllithium (1.6 M in hexane, 12.8 mmol) over 10 min under argon at −78 °C. The mixture was stirred for 1.5 h below −70 °C and then allowed to warm slowly to 5 °C over 1 h. The solution was cooled to −78 °C again and then treated via a syringe with a solution of **3** (1.5 g, 10.8 mmol) in dry THF (10 mL). The mixture was allowed to warm slowly to room temperature overnight, poured into 20% sulfuric acid (100 mL), and stirred for 3 h. The solution was extracted with ethyl acetate. The combined extracts were washed with H_2O, saturated sodium bicarbonate solution, and brine; dried over anhydrous sodium sulfate; and evaporated to give a light amber oil. This oil was crystallized in ether/hexane (1:1) to provide 2.85 g (90%) of (*S*)-(1-phenylsulfonylindol-2-yl)propan-2-ol **4** as white crystals, mp 88−89 °C. $[\alpha]^{D}_{295}$ −56.11° (*c* 0.875, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.17−8.16 (m, 9H), 6.51 (d, J = 0.76 Hz, 1H), 4.26 (m, 1H), 3.25, 3.01 (m, 2H), 1. 91 (s, br, 1H), 1.30 (d, J = 6.2 Hz, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 138.8, 138.5, 137.4, 133.8, 129.7, 129.3, 126.3, 124.4, 123.9, 120.5, 115.1, 111.6, 67.2, 39.1, 23.1. MS (CI, NH_3): *m/e* 316 (MH^+, 29.4), 298 (11.6), 271 (40.4). HRMS (FAB, glycerol): *m/e* calcd for $C_{17}H_{18}NO_3S$ [MH^+] 316.10074, found 316.10084.

(*S*)-Indol-2-ylpropan-2-ol (5a). A solution of **4** (2.85 g, 9.0 mmol) in 50 mL of methanol/water (3:1) containing KOH (2.5

(15) A mixture of dry dichloromethane and acetonitrile (1:10) was used as eluting solvent. Using ethyl acetate instead of acetonitrile gave very low yield of **7b**. The trace of acids in ethyl acetate probably accelerated the decomposition of **7b**.

(16) The snake venom phosphodiesterase and P1 nuclease digestion and HPLC analysis were carried out at ISIS Pharmaceuticals (Carlsbad, CA) by Dr. M. Manoharan.

(17) Wang, J.-C.; Just, G.; Guzaev, A. P.; Manoharan, M. *J. Org. Chem.* **1999**, *64*, 2595.

g, 44.6 mmol) was refluxed for 5 h and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine, dried over anhydrous sodium sulfate, and evaporated to afford 1.65 g (95%) of **5a** as a light amber oil. $[\alpha]^{D}_{295}$ 9.12° (*c* 1.035, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.51 (s, 1H), 7.58−7.05 (m, 4H), 6.28 (s, 1H), 4.10 (m, 1H), 2.93, 2.76 (m, 2H), 2.06 (s, br, 1H), 1.25 (d, J = 6.2 Hz, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.6, 136.2, 128.9, 121.3, 119.9, 119.7, 110.7, 100.9, 68.0, 37.5, 23.3. MS (CI, NH_3): *m/e* 176 (MH^+, 47.0), 175 (M^+, 62.6), 130 (100.0). HRMS (FAB, glycerol): *m/e* calcd for $C_{11}H_{14}NO$ [MH^+] 176.107539, found 176.10746.

Indolyl-oxazaphosphorine (7a). A dried 25-mL round-bottomed flask containing 10 mL of dry CH_3CN was flushed with argon and sealed with a septum, and then 100 μL of PCl_3 (1.15 mmol) was added via a microsyringe. The flask was cooled to 0 °C, and a solution of **5a** (200 mg, 1.15 mmol) in CH_3CN (0.35 mL) containing triethylamine (525 μL, 3.8 mmol) was introduced. The reaction mixture was stirred for 30 min at 0 °C and then warmed to 60 °C for 10 h. The flask was cooled to −78 °C, and a solution of 5′-*O*-TBDMS-thymidine (410 mg, 1.15 mmol) in CH_2Cl_2 (0.4 mL) was added. The reaction mixture was stirred for 30 min. Triethylammonium chloride was filtered off and washed with CH_2Cl_2. The filtrate was concentrated and purified with flash chromatography (CH_2Cl_2/CH_3CN 1:10) to give 346 mg (54%) of white solid indolyl-oxazaphosphorine **7a**, mp 80−82 °C. Two diastereoisomers of indol-oxazaphosphorine **7a** were obtained in a ratio of 9:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 121.56 (12.4%), 120.67 (87.6%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.81 (s, br, 1H), 7.39 (s, 1H), 7.54, 7.17 (m, 4H), 6.36 (dd, J = 9.0, 5.5 Hz, 1H), 6.33 (s, 1H), 4.72 (m, 1H), 4.41 (m, 1H), 3.94 (m, 1H), 3.58 (m, 1H), 3.06−3.10 (m, 3H), 2.36 (m, 1H), 1.97 (m, 1H), 1.87 (s, 3H), 1.48 (d, J = 5.5 Hz, 3H), 0.84 (s, 9H), −0.05 (ss, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.7, 150.2, 137.8, 137.5, 136.4, 136.3, 135.2, 129.8 (d, J = 2.1 Hz), 122.2, 121.5, 120.4, 111.0, 110.6, 110.4, 103.2, 86.1 (d, J = 3.1 Hz), 84.7, 73.6 (d, J = 3.6 Hz), 71.6, 71.5, 62.9, 40.3 (d, J = 2.1 Hz), 26.0, 25.9, 22.9 (d, J = 3.6 Hz), 18.3, 12.5, −5.4, −5.7. HRMS (FAB, glycerol): *m/e* calcd for $C_{27}H_{39}N_3O_6$-SiP [MH^+] 560.234578, found 560.234590.

Phosphorothioate Triester (9a). To a dried 5-mL round-bottomed flask was added indolyl-oxazaphosphorine **7a** (50 mg, 85 μmol) and 3′-*O*-TBDPS-thymidine ($T^{5'}OH$) (40.8 mg, 85 μmol). The flask was flushed with argon and sealed with a septum, and then 2 mL of dry $CHCl_3$ was added followed by DBU (14 μL, 94 μmol). The reaction mixture was stirred at room temperature overnight, passed though a short silica gel column to filter off DBU, and eluted with dried CH_2Cl_2/CH_3-CN (1:1). The filtrate was evaporated to afford a colorless oil. The oil was redissolved in dry CH_2Cl_2 (5 mL), and Beaucage's reagent (30 mg, 1.5 mmol) was added. Evaporation of the solvent followed by flash chromatography (dichloromethane/acetone 5:1) afforded 71 mg (78%) of white solid phosphorothioate triester **9a**, mp 115−116 °C. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.76 (98.65%), 66.59(1.35%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.93 (s, 1H), 9.31 (s, 1H), 8.85 (s, 1H), 7.62−6.93 (m, 16H), 6.46 (dd, J = 8.0, 6.0 Hz, 1H), 6.26 (s, 1H), 6.05 (dd, J = 9.2, 5.5 Hz, 1H), 4.92 (m, 1H), 4.76 (m, 1H), 4.31 (m, 1H), 4.03 (m, 1H), 3.82 (m, 1H), 3.80, 3.50 (m, 2H), 3.67, 3.58 (m, 2H), 3.00 (m, 2H), 2.31 (m, 1H), 1.94 (s, 3H), 1.90 (s, 3H), 1.85 (m, 1H), 1.60 (m, 1H), 1.26 (d, J = 6.0 Hz, 3H), 1.14 (m, 1H), 1.80 (s, 9H), 0.89 (s, 9H), 0.07 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.83, 163.80, 150.7, 150.3, 135.5 (d, J = 3.6 Hz), 134.5, 134.1, 132.7, 132.5, 130.1, 130.0, 128.4, 127.9, 127.8, 120.9, 119.5, 119.3, 111.2, 110.4, 100.6, 85.2 (d, J = 8.1 Hz), 85.1 (d, J = 9.1 Hz), 84.8, 84.2, 79.7 (d, J = 2.6 Hz), 76.8, 73.3, 66.8 (d, J = 6.4 Hz), 63.0, 40.1, 37.5, 36.0 (d, J = 9.1 Hz), 26.6, 25.7, 21.2, 18.8, 18.1, 12.4, 12.3, −5.61, −5.56. MS (FAB, NBA): *m/e* 1072 (MH^+, 1.3).

(*R*)-Glycidyl *tert*-Butyldimethylsilyl Ether (12). To a solution of (*S*)-glycidol (10 g, 135 mmol, 98% ee from Aldrich Chemical Co.) in dichloromethane (60 mL) containing triethylamine (20 mL, 144 mmol) were added a solution of TBDMSCl (22.4 g, 148 mmol) in dichloromethane (50 mL) and DMAP (0.6 g, 5.4 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5 h. Triethylammonium chloride was filtered off and washed with dichloromethane. The filtrate was washed with brine, dried over anhydrous sodium sulfate, and concentrated. The residue was passed through a short silica gel column to remove polar impurities and eluted with hexane/ethyl acetate (3:2). After removal of the solvent, a light yellow oil was collected and distilled under vacuum (55−60 °C/3 mmHg) to provide 19 g (75%) of colorless liquid **12**. $[\alpha]^{D}_{295}$ −6.09° (*c* 6.47, ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.84, 3.65 (m, 2H), 3.08 (m, 1H), 2.76, 2.63 (m, 2H), 0.88 (s, 9H), 0.07 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 63.5, 52.2, 44.27, 25.6, 18.1, −5.51, −5.55. MS (CI, NH_3): *m/e* 206 (MNH_4^+, 12.1), 189 (MH^+, 12.0), 131 (100.0).

(*S*)-Glycidyl *tert*-butyldimethylsilyl ether [(*S*)-12] was obtained from (*R*)-glycidol in 81% yield as described for **12**. $[\alpha]^{D}_{295}$ +6.11° (c 2.75, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.81, 3.61 (m, 2H), 3.04 (m, 1H), 2.72, 2.59 (m, 2H), 0.86 (s, 9H), 0.036 (ss, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 63.78, 52.44, 44.45, 25.90, 18.38, −5.28, −5.32. MS (CI, NH_3): 206 (MNH_4^+, 3.4), 189 (MH^+, 18.2), 131 (78.5), 74 (100.0).

(*R*)-1-*tert*-Butyldimethylsilyloxy-3-(1-phenylsulfonylindol-2-yl)propan-2-ol (13). To a solution of 1-phenylsulfonylindole (6.2 g, 24 mmol) in dry THF (60 mL) was added dropwise 18 mL of *n*-butyllithium (1.6 M in hexane, 28.8 mmol) over 10 min under argon at −78 °C. The mixture was stirred for 1.5 h below −70 °C and then allowed to warm slowly to 5 °C over 1 h. The solution was cooled to −78 °C again, and a solution of **12** (4.5 g, 24 mmol) in dry THF (10 mL) was added. The mixture was allowed to warm slowly to room temperature overnight and poured into saturated NH_4Cl solution (80 mL). The mixture was extracted with ethyl acetate. The combined extracts were washed with H_2O, saturated sodium bicarbonate solution, and brine; dried over anhydrous sodium sulfate; and evaporated to afford a deep red oil. This oil was purified by flash chromatography (ethyl acetate/hexane 1:1) to provide 5.2 g (48%) of pale light yellow solid **13**, mp 77−78.5 °C. $[\alpha]^{D}_{295}$ −26.90° (*c* 0.875, ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.20−8.16 (m, 9H), 6.57 (s, 1H), 4.15 (m, 1H), 3.74, 3.59 (m, 2H), 3.24, 3.10 (m, 2H), 2.42 (s, broad, 1H), 0.93 (s, 9H), 0.105 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 138.7, 138.1, 137.1, 133.4, 129.6, 129.0, 126.0, 124.0, 123.5, 120.2, 114.7, 111.0, 70.6, 66.3, 32.8, 25.71, 18.12, −5.50, −5.54. MS (FAB, NBA): *m/e* 446 (MH^+, 53.5). HRMS (FAB, glycerol): *m/e* calcd for $C_{23}H_{32}NO_4SiS$ [MH^+] 446.18213, found 446.18232.

(*S*)-1-*tert*-Butyldimethylsilyloxy-3-(1-phenylsulfonylindol-2-yl)propan-2-ol [(*S*)-13] was obtained from (*S*)-**12** in 40% yield as described for **13**, mp 78−79.5 °C. $[\alpha]^{D}_{295}$ +26.88° (*c* 1.09, ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19−8.16 (m, 9H), 6.57 (s, 1H), 4.14 (m, 1H), 3.74, 3.58 (m, 2H), 3.23, 3.09 (m, 2H), 2.57 (d, 1H, ^3J = 4.5 Hz), 0.93 (s, 9H), 0.10 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 138.76, 138.13, 137.11, 133.49, 129.64, 129.04, 126.01, 124.01, 123.55, 120.20, 114.75, 111.06, 70.69, 66.32, 32.84, 25.72, 18.12, −5.50, −5.54. MS (CI, NH_3): 446 (MH^+, 72.4), 388 (40.0), 247 (80.0), 130 (100.0).

(*R*)-3-Indol-2-yl-propane-1,2-diol [(*R*)-14]. A solution of **13** (4.5 g, 10.1 mmol) in 50 mL of methanol/water (3:1) containing KOH (2.8 g, 50 mmol) was refluxed for 5 h and extracted with ethyl acetate. The combined extracts were washed with H_2O and brine, dried over anhydrous sodium sulfate, and evaporated to afford 1.68 g (87%) of pale solid (*R*)-**14**, mp 57.5−58.5 °C. $[\alpha]^{D}_{295}$ +10.8° (*c* 1.0, ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.54 (s, 1H), 7.54−7.12 (m, 4H), 6.21 (s, 1H), 3.82 (m, 1H), 3.52, 3.34 (m, 2H), 3.28 (s, broad, 2H), 2.72 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 135.9, 135.4, 128.2, 121.2, 119.7, 119.5, 110.5, 100.64, 71.5, 65.7, 31.6. MS (EI): *m/e* 191 ($M^{+\bullet}$, 40.8), 130 (100.0). HRMS (EI): *m/e* calcd for $C_{11}H_{13}NO_2$ [M^+] 191.09462, found 191.09468.

(*S*)-3-Indol-2-yl-propane-1,2-diol [(*S*)-14] was obtained from (*S*)-**13** in 98% yield as described for (*R*)-**14**, mp 58.5−60 °C. $[\alpha]^{D}_{295}$ −8.53° (*c* 0.92, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.58 (s, 1H), 7.53−7.00 (m, 4H), 6.21 (s, 1H), 3.88 (m, 1H), 3.56, 3.40 (m, 2H), 3.2 (s, broad, 1H), 2.79 (m, 2H), 2.00 (s, broad, 1H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.24,

135.71, 128.46, 121.45, 119.96, 119.78, 110.75, 100.93, 71.80, 66.06, 31.8. MS (CI, NH_3): 192 (MH^+, 100.0), 130 (68.6).

(*R*)-3-Indol-2-yl-2-hydroxypropyl *p*-Toluenesulfonate [(*R*)-15]. To a solution of (*R*)-**14** (15 g, 78.5 mmol) in dry pyridine (120 mL) was added *p*-toluenesulfonyl chloride (15.5 g, 81.3 mmol) at 0 °C. After stirring for 5 h at 0 °C, the solution was poured into 100 mL of cold HCl (6 N) and extracted with ether. The combined extracts were washed with HCl (6 N) and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified by flash chromatography (hexane/ethyl acetate 1:5) to give 24.5 g (90%) of white solid (*R*)-**15**, mp 112−113 °C. $[\alpha]^D_{295}$ −1.03° (c 1.035, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.52 (s, br, 1H), 7.77−7.03 (m, 8H), 6.19 (s, 1H), 4.15 (m, 1H), 3.97 (m, 2H), 2.92 (m, 2H), 2.71 (d, J = 4.5 Hz, 1H), 2.42 (s, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 145.4, 136.3, 134.2, 132.4, 130.1, 128.3, 128.0, 121.5, 119.9, 119.7, 110.8, 101.4, 72.7, 69.2, 31.5, 21.7. MS (FAB, NBA): *m/e* 346 (MH^+, 80.5). HRMS (FAB, glycerol): *m/e* calcd for $C_{18}H_{20}NO_4S$ [MH^+] 346.11130, found 346.11123.

(*S*)-3-Indol-2-yl-2-hydroxypropyl *p*-toluenesulfonate [(*S*)-15] was obtained from (*S*)-**14** in 89% yield as described for (*R*)-**15**, mp 96−97 °C. $[\alpha]^D_{295}$ +0.93° (c 1.00, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.51 (s, br, 1H), 7.78−7.06 (m, 8H), 6.21 (s, 1H), 4.16 (m, 1H), 4.00 (m, 2H), 2.97 (m, 2H), 2.44 (s, 3H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 145.1, 136.0, 133.9, 132.1, 129.8, 128.0, 127.7, 121.3, 119.7, 119.5, 110.5, 101.2, 72.4, 68.9, 31.2, 21.4. MS (CI, NH_3): *m/e* 346 (MH^+, 6.0), 174 (100).

(*R*)-3-Hydroxy-4-(2-indolyl)butyronitrile (5b). To a solution of LiCN (0.5 M in DMF, 30 mL) were added (*R*)-**15** (5.6 g, 16.2 mmol) and sodium cyanide (1.5 g, 30.6 mmol). The reaction mixture was stirred for 1 h at 100 °C, cooled to room temperature, poured into 80 mL of ice water, and extracted with ethyl acetate. The combined extracts were washed with saturated sodium bicarbonate and brine, dried over anhydrous sodium sulfate, and evaporated to yield a deep red oil. This oil was purified by flash chromatography (hexane/ethyl acetate 2:3) to give 2.4 g (74%) of light yellow solid **5b**.

The chiralty of **5b** was analyzed by HPLC (Varian Vista 5500) with Chiralcel OD column (4.6 mm × 250 mm) with 1.5 mL/min flow rate of hexane/ethanol (9:1) and was 91% ee. Compound **5b** (2.0 g) was dissolved in chloroform (2 mL), and the solvent was slowly evaporated at room temperature in atmosphere. After a week, crystals were formed and filtered off, mp 92−93 °C. The filtrate was collected and dried under vacuum to give 0.5 g of a light amber oil **5b** in 96% ee. $[\alpha]^D_{295}$ −8.67° (*c* 0.565, ethyl acetate). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (s, br, 1H), 7.5−7.0 (m, 4H), 6.32 (s, 1H), 4.22 (m, 1H), 3.05, 2.96 (m, 2H), 2.67 (s, br. 1H), 2.49 (m, 2H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 136.1, 133.3, 128.0, 121.7, 119.9, 119.8, 110.6, 101.8, 117.2, 67.2, 34.7, 25.0. MS (EI): *m/e* 200 ($M^{+\bullet}$, 48.5), 130 (100). HRMS (FAB, glycerol): *m/e* calcd for $C_{12}H_{13}N_2O$ [MH^+] 201.10278, found 201.10285.

(*S*)-3-Hydroxy-4-(2-indolyl)butyronitrile [(*S*)-5b]. By the same procedure as described for **5b**, (*S*)-**5b** was obtained from (*S*)-**15** in 64% yield. After crystallization in chloroform for 2 weeks, a red oil (*S*)-**15** in 96% ee was obtained from the mother liquor (optical rotation could not be detected because of the deep color). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.42 (s, br, 1H), 7.0−7.5 (m, 4H), 6.30 (d, J = 1.48 Hz, 1H), 4.20 (m, 1H), 3.01 (m, 2H), 2.47, 2.49 (m, 2H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.56, 133.58, 128.55, 121.94, 120.16, 120.06, 110.74, 102.12, 117.12, 67.55, 35.10, 25.28. MS (EI): 200 ($M^{+\bullet}$, 59.6), 130 (100.0).

(*S*)-3-Indol-2-yl-1-isopropylamino-2-propanol (16). To a pressure vessel were added 2.94 g of (*S*)-**15** and 10 mL of isopropylamine. The mixture was stirred overnight at 110 °C. Evaporation of the solvent afforded an amber oil, which was purified by flash chromatography (acetone/triethylamine 10:1) to give 1.6 g (81%) of a sticky oil **16**. $[\alpha]^D_{295}$ −8.13° (*c* 0.75, ethyl acetate). ^{1}H NMR (270 MHz, CDCl3): δ 9.01 (s, br, 1H), 7.0−7.5 (m, 4H), 6.23 (s, 1H), 3.89 (m, 1H), 2.72−3.03 (m, 5H), 2.44, 2.59 (m, 2H), 1.05 (d, J = 6.18 Hz, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.6, 136.2, 128.4, 121.1, 119.8, 119.4, 110.7, 100.6, 69.3, 51.8, 49.0, 33.3, 23.1, 22.8. MS (CI, NH_3): 233 (MH^+, 100.0), 130 (31.9). HRMS (FAB, glycerol): *m/e* calcd for $C_{14}H_{21}N_2O$ [MH^+] 233.16538, found 233.16539.

(*S*)-*N*-(3-Indol-2-yl-2-hydroxy)-propyl-*N*-isopropylacetamide (5c). To a solution of **16** (0.2 g, 0.86 mmol) in dry CH_3CN (20 mL) was added acetic anhydride (0.1 mL, 1.06 mmol). The mixture was stirred for 4 h at room temperature, washed with saturated sodium bicarbonate solution and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate) to give 0.22 g (92%) of light yellow solid **5c**, mp 122−123 °C. $[\alpha]^D_{295}$ −13.56° (*c* 0.78, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 9.17 (s, br, 1H), 7.0−7.5 (m, 4H), 6.25 (s, 1H), 3.98 (m, 2H), 3.50, 3.10 (m, 2H), 2.94 (m, 2H), 2.15 (s, 3H),1.14, 1.12 (d, J = 6.42 Hz, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 173.5, 136.3, 136.1, 128.2, 121.1, 119.7, 119.4, 110.8, 100.8, 73.4, 50.1, 47.9, 34.1, 21.9, 21.3, 20.7. MS (CI, NH_3): *m/e* 275 (MH^+, 100.0), 257 (75.0), 256 (74.1). HRMS (FAB, glycerol): *m/e* calcd for $C_{16}H_{23}N_2O_2$ [MH^+] 275.17595, found 275.17602.

(*S*)-1-*tert*-Butyldimethylsilyloxy-3-(indol-2-yl)-2-propanol (5d). To a solution of (*S*)-**14** (1.3 g, 6.8 mmol) in dry dichloromethane (30 mL) containing triethylamine (1.1 mL, 7.9 mmol) were added a solution of TBDMSCl (1.16 g, 7.7 mmol) in dichloromethane (5 mL) and DMAP (34.2 mg, 0.28 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 5 h. Triethylammonium chloride was filtered off and washed with dichloromethane. The filtrate was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give a light red oil. Purification by flash chromatography (ethyl acetate) afforded 1.57 g (74%) of an amber oil **5d**. $[\alpha]^D_{295}$ +25.42° (*c* 1.150, ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.74 (br. s, 1H), 7.55−7.06 (m, 4H), 6.24 (s, 1H), 3.99 (m, 1H), 3.65, 3.49 (m, 2H), 2.92(m, 2H), 2.74 (d, J = 3.4 Hz, 1H), 0.91 (s, 9H), 0.085 (s, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 136.3, 136.2, 128.4, 121.2, 119.8, 119.5, 110.6, 100.7, 71.7, 66.5, 31.5, 25.9, 18.3, −5.24, −5.27. MS (FAB, NBA): *m/e* 306 (MH^+, 22.0). HRMS (FAB, glycerol): *m/e* calcd for $C_{17}H_{28}NO_2Si$ [MH^+] 306.18893, found 306.18895.

Indolyl-oxazaphosphorine (7b). A dried 5-mL round-bottomed flask containing 1 mL of dry THF was flushed with argon and sealed with a septum, and then PCl_3 (44 μL, 0.5 mmol) was introduced via a microsyringe. The flask was cooled to −78 °C, and a solution of **5b** (100 mg, 0.5 mmol) in THF (1 mL) containing triethylamine (0.3 mL, 2.2 mmol) was added via a syringe. The reaction mixture was stirred for 30 min at −78 °C, and then warmed to 0 °C for 1 h. The flask was cooled to −78 °C again, and a solution of 5-*O*′-TBDMS-thymidine (178 mg, 0.5 mmol) in THF (0.5 mL) was added via a syringe. The reaction mixture was stirred at −78 °C for 30 min, and then the solution was warmed to room temperature. Triethylammonium chloride was filtered off and washed with CH_2Cl_2. The filtrate was concentrated and purified with TLC chromatography (CH_2Cl_2/CH_3CN 1:5) to afford 94 mg (32%) of white solid indolyl-oxazaphosphorine **7b**, mp 85−86 °C. Two diastereoisomers of **7b** were obtained in a ratio of 30:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 120.78 (96.8%), 120.65 (3.2%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (s, br, 1H), 7.39 (s, 1H), 7.57−7.17 (m, 4H), 6.42 (s, 1H), 6.32 (dd, J = 8.8, 5.0 Hz, 1H), 4.80 (m, 1H), 4.52 (m, 1H), 4.02 (m, 1H), 3.65, 3.23 (m, 2H), 3.40, 3.25 (m, 2H), 2.91, 2.82 (m, 2H), 2.38, 2.04 (m, 2H), 1.87 (s, 3H), 0.85 (s, 9H), −0.004 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.3, 149.9, 137.6, 137.5, 134.9, 133.3, 129.4 (d, J = 1.8 Hz), 122.5, 121.6, 120.4, 115.6, 110.8, 110.2 (d, J = 11.0 Hz), 104.2, 85.7 (d, J = 2.7 Hz), 84.4, 74.4 (d, J = 6.4 Hz), 69.4 (d, J = 7.2 Hz), 62.6, 39.9 (d, J = 1.8 Hz), 30.6 (d, J = 4.6 Hz), 25.6, 25.4 (d, J = 2.6 Hz), 18.0, 12.2, −5.6, −5.9. MS (FAB, NBA): *m/e* 585 (MH^+, 19.7). HRMS (FAB, glycerol): *m/e* calcd for $C_{28}H_{38}N_4O_6PSi$ [MH^+] 585.22982, found 585.22985.

Sp-Indolyl-oxazaphosphorine (7Sb). By the same procedure as described for **7b**, white solid (*S*)-**5b** was obtained from (*S*)-**5b** in 21% yield after purification by flash chromatography (CH_2Cl_2/CH_3CN 1:10), mp 93−94 °C. Two diastereoisomers of indolyl-oxazaphosphorine **7Sb** were obtained in a

ratio of 6:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 120.53 (85.3%), 120.72 (14.7%). The following NMR spectra were assigned for the major isomer. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.20 (s, br, 1H), 7.42 (s, 1H), 7.57−7.17 (m, 4H), 6.42 (s, 1H), 6.34 (dd, J = 8.8, 5.0 Hz, 1H), 4.82 (m, 1H), 4.47 (m, 1H), 3.94 (m, 1H), 3.76, 3.66 (m, 2H), 3.40−3.24 (m, 2H), 2.91 (m, 2H), 2.41, 2.01 (m, 2H), 1.89 (s, 3H), 0.88 (s, 9H), 0.066 (ss, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.66, 150.41, 138.11, 137.87, 135.14, 133.60, 129.67, 122.78, 121.86, 120.75, 116.13, 111.18, 110.51 (d, J = 10.8 Hz), 104.41, 86.41 (d, J = 4.1 Hz), 84.81, 75.49 (d, J = 12.9 Hz), 70.71 (d, J = 8.2 Hz), 63.15, 39.82, 30.88 (d, J = 5.7 Hz), 25.96, 25.66, 18.36, 12.55, −5.30, −5.41. MS (FAB, NBA): 557 (M − HCN, 44.5).

Indolyl-oxazaphosphorine (7Sc). By the same procedure as described for **7b**, 42 mg (58%) of **7Sc** was obtained from **5c** (30 mg, 0.11 mmol). Two diastereoisomers of **7Sc** were obtained in a ratio of 6.8:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 120.76 (12.8%), 120.68 (87.2%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, br, 1H), 7.39 (s, 1H), 7.54−7.17 (m, 4H), 6.35 (m, 2H), 4.74 (m, 1H), 4.46 (m, 1H), 4.10 (m, 2H), 3.89, 3.47 (m, 3H), 3.11 (m, 1H), 2.4, 1.9 (m, 2H), 2.17 (s, 3H), 1.86 (s, 3H), 1.24 (m, 6H), 0.87 (s, 9H), 0.035 (ss, 6H).

Indolyl-oxazaphosphorine (7Sd). By the same procedure as described for **7b**, 15 mg (22%) of white solid **7Sd** was obtained from **5d** (60 mg, 0.20 mmol), mp 70−71 °C. Two diastereoisomers of **7Sd** were obtained in a ratio of 14:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 121.02 (6.8%), 120.64 (93.2%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.30 (s, br, 1H), 7.38 (d, J = 1.5 Hz), 7.54−7.13 (m, 4H), 6.36 (m, 2H), 4.75 (m, 1H), 4.42 (m, 1H), 3.87 (m, 2H), 3.80 (m, 1H), 3.72 (m, 1H), 3.16 (m, 3H), 2.29 (m, 1H), 1.89 (m, 1H), 1.85 (d, J = 1.0 Hz, 3H), 0.90, 0.84 (ss, 18H), 0.097(ss, 12H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 163.25, 149.82, 137.78, 137.66, 135.62, 134.98, 129.63, 122.04, 121.28, 120.22, 110.75, 110.20 (d, J = 10.9 Hz), 103.38, 86.23, 84.47, 75.68 (d, J = 7.4 Hz), 73.79 (d, J = 10.0 Hz), 66.08, 62.70, 39.71, 29.52, 28.45 (d, J = 5.5 Hz), 28.02, 25.71, 18.21 (d, J = 11.9 Hz), 12.28, −5.45, −5.56, −5.69. MS (FAB, NBA): 690 (MH^+, 2.76), 689 (M^+, 6.21).

Phosphorothioate Triester (9b). To a round-bottomed flask was added **7b** (80 mg, 0.137 mmol) and 3′-*O*-TBDPS-thymidine (50 mg, 0.104 mmol). The flask was flushed with argon and sealed with a septum. Dry THF (0.5 mL) was introduced followed by DBU (40 μL, 0.268 mmol) via a syringe. This reaction mixture was shaken at room temperature for 10 min and passed though a short silica gel column to remove DBU, and the column was eluted with CH_3CN. The solvent was evaporated to afford light yellow solid. This solid was redissolved in dry CH_2Cl_2 (2 mL), and Beaucage's reagent (35 mg, 0.175 mmol) was added. After 5 min, evaporation of the solvent followed by flash chromatography (dichloromethane/acetone 5:1) afforded 84 mg (74%) of light yellow solid phosphorothioate triester **9b**, mp 113−114 °C. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.55 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.21 (s, br, 1H), 9.56 (s, br, 1H), 9.14 (s, br, 1H), 7.62−6.92 (m, 16 H), 6.35 (m, 1H), 6.28 (s, 1H), 6.06 (dd, J = 9.0, 5.5 Hz, 1H), 4.88 (m, 1H), 4.82 (m, 2H), 4.32 (m, 1H), 4.06 (m, 1H), 3.93 (m, 1H), 3.88 (m, 1H), 3.71 (m, 2H), 3.63 (m, 1H), 3.21 (m, 2H), 2.61 (m, 2H), 2.29, 1.95 (m, 2H), 1.90 (s, 3H), 1.88 (s, 3H), 1.82, 1.40 (m, 2H), 1.06 (s, 9H), 0.88 (s, 9H), 0.063 (ss, 6H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 164.13, 164.09, 150.70, 150.35, 135.82, 135.65, 135.54, 134.65, 132.75, 132.51, 131.97, 130.12 (d, J = 3.6 Hz), 128.32, 127.93, 127.89, 121.45, 119.76, 119.56, 115.63, 111.22 (d, J = 2.7 Hz), 110.80, 101.26, 85.24 (d, J = 8.1 Hz), 84.94, 84.76 (d, J = 9.1 Hz), 84.44, 80.32, 74.02 (d, J = 4.6 Hz), 72.55, 67.22 (d, J = 5.5 Hz), 63.02, 39.89, 38.16, 33.18 (d, J = 6.4 Hz), 26.67, 25.75, 23.45, 18.81, 18.12, 12.41 (d, J = 7.2 Hz), −5.5, −5.6. HRMS (FAB, NBA/CsI): *m/e* calcd for $C_{54}H_{69}N_6O_{11}Si_2PSCs$ [MCs^+] 1229.30755, found 1229.30710.

Phosphorothioate Triester (9Sb). By the same procedure as described for **9b**, 388 mg (68%) of white solid **9Sb** was obtained from the reaction of **7Sb** (307 mg, 0.526 mmol) and 3′-*O*-TBDPS-thymidine (253 mg, 0.526 mmol), mp 116−117 °C. Two diastereoisomers of **9Sb** were obtained in a ratio of 24:1 as established by ^{31}P NMR. ^{31}P NMR (202.3 MHz, $CDCl_3$): δ 66.31 ppm (96%), 65.73 (4%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.16 (s, br, 1H), 9.13 (s, br, 1H), 8.60 (s, br, 1H), 7.63−7.05 (m, 16H), 6.33 (s, 1H), 6.27 (m, 1H), 6.17 (dd, J = 9.0, 5.0 Hz, 1H), 4.91 (m, 2H), 4.30 (m, 1H), 4.10 (m, 1H), 3.88 (m, 1H), 3.79 (m, 2H), 3.54, 3.45 (m, 2H), 3.19 (m, 2H), 2.65 (m, 2H), 2.28 (m, 2H), 2.01 (m, 1H), 1.89 (s, 3H), 1.86 (s, 3H), 1.87 (m, 1H), 1.06 (s, 9H), 0.88 (s, 9H), 0.068 (ss, 6H). ^{13}C NMR (67.9 MHz, $CDCl_3$): δ 163.82, 163.71, 150.35, 150.27, 136.33, 136.10, 135.79, 134.85, 132.96, 132.81, 131.61, 130.30, 128.33, 128.13, 128.06, 122.20, 120.24, 115.85, 111.36 (d, J = 7.7 Hz), 110.94, 102.61, 86.32, 85.49 (d, J = 6.2 Hz), 85.13 (d, J = 8.2 Hz), 84.60, 80.75 (d, J = 4.6 Hz), 77.29, 74.23 (d, J = 5.1 Hz), 73.06, 67.86 (d, J = 6.7 Hz), 63.16, 39.92, 38.92 (d, J = 4.1 Hz), 33.57 (d, J = 4.6 Hz), 31.00, 26.90, 25.96, 23.65 (d, J = 4.6 Hz), 19.06, 18.34, 12.58 (d, J = 4.6 Hz), −5.33, −5.41. MS (FAB, NBA): 1097 (MH^+, 11.1), 759 (5.4), 377 (28.1), 339 (51.7), 182 (100).

Phosphorothioate Triester (9Sd). By the same procedure as described for **9b**, 18 mg (70%) of **9Sd** was obtained from **7Sd** (15 mg, 0.022 mmol). Triester **9Sd** was directly used in the next step to test the removal of silyloxyl chiral auxiliary in the presence of TBAF. ^{31}P NMR (109.4 MHz, $CDCl_3$): δ 69.10 ppm.

Dithymidinyl Phosphorothioate (Sp-10). To a solution of **9b** (60 mg, 0.056 mmol) in methanol (1 mL) was added 20 mL of aqueous ammonia (28%). The solution was stirred at 50 °C for 30 min, neutralized with HCl (6 N), and extracted with ethyl acetate. The combined extracts were dried over anhydrous sodium sulfate. The solvent was evaporated, and the residue was purified by flash chromatography (acetone/triethylamine 10:1) to give 32 mg (58%) of Sp-**10** which existed as a triethylammonium salt. ^{31}P NMR (202.3 MHz, CD_3OD): δ 58.98 ppm. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (d, J = 1.0 Hz, 1H), 7.56−7.31 (m, 10H), 7.48 (d, J = 1.0 Hz, 1H), 6.39 (dd, J = 9.2, 5.0 Hz, 1H), 6.05 (dd, J = 8.8, 5.0 Hz, 1H), 4.88 (m, 1H), 4.47 (m, 1H), 3.98 (m, 1H), 3.95 (m, 1H), 3.74, 3.50 (m, 2H), 3.67 (m, 2H), 2.86, 1.10 (q, t, $N(CH_2CH_3)_3$), 2.28 (m, 1H), 2.00 (m, 3H), 1.83 (d, J = 1.0 Hz, 3H), 1.76 (d, J = 1,0 Hz, 3H), 0.98 (s, 9H), 0.80 (s, 9H), 0.009 (ss, 6H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.3, 166.2, 152.4, 151.9, 138.0, 137.2, 136.8, 136.8, 134.3, 134.2, 131.1, 131.0, 129.0, 128.9, 112.2, 111.0, 88.0 (d, J = 9.1 Hz), 87.6 (d, J = 4.6 Hz), 86.3, 86.1, 77.9 (d, J = 5.5 Hz), 76.3, 66.4 (d, J = 6.4 Hz), 64.8, 47.4 (NEt_3), 41.3, 40.5 (d, J = 4.5 Hz), 27.2, 26.4, 19.6, 19.1, 12.6, 12.5, 9.7 (NEt_3), −5.22, −5.28. MS (FAB, NBA): *m/e* 937 (MNa^+).

Phosphorothioate (Sp-11). A solution of TBAF (1.0 M in DMF, 4 mL) containing dimer Sp-**10** (20 mg, 0.017 mmol) was stirred at room temperature for 1 h. The solvent was evaporated under vacuum, and the residue was purified by flash chromatography (acetone/triethylamine 1:1) to give 15 mg (95%) of Sp-**11**, which existed as a tetrabutylammonium salt. ^{31}P NMR (202.3 MHz): δ 58.76 ppm (CD_3OD), 55.45 ppm (D_2O). ^{1}H NMR (500 MHz, CD_3OD): δ 7.80 (d, J = 1.0 Hz, 1H), 7.78 (d, J = 1.5 Hz), 6.29 (dd, J = 8.0, 6.0 Hz, 1H), 6.22 (dd, J = 8.0, 6.0 Hz, 1H), 4.98 (m, 1H), 4.45 (m, 1H), 4.12 (m, 1H), 4.09, 3.98 (m, 2H), 3.98 (m, 1H), 3.74 (m, 2H), 3.17, 1.59, 1.35, 0.94 (NBu_4^+), 2.40 (m, 1H), 2.20 (m, 2H), 2.12 (m, 1H), 1.89 (d, J = 1.0 Hz, 3H), 1.80 (d, J = 1.0 Hz, 3H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.4, 166.3, 152.4, 152.2, 138.08, 138.06, 112.0, 111.5, 87.6 (d, J = 5.5 Hz), 87.3 (d, J = 10.0), 86.1, 86.0, 77.0 (d, J = 5.5 Hz), 72.8, 66.6 (d, J = 5.4 Hz), 62.7, 59.39 (t, NBu_4^+), 40.7, 40.0 (d, J = 4.5 Hz), 24.69 (NBu_4^+), 20.61 (NBu_4^+), 13.85 (NBu_4^+), 12.6, 12.3. HRMS (FAB): *m/e* calcd for $C_{20}H_{27}N_4O_{11}PSNa$ [MNa^+] 585.10323, found 585.10340.

Dithymidinyl phosphorothioate (Rp-10) was obtained from **9Sb** in 80% yield as described for Sp-**10**. ^{31}P NMR (202.3 MHz, CD_3OD): δ 59.07 ppm. The minor isomer could not be identified from its ^{31}P NMR. ^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (d, J = 1.0 Hz, 1H), 7.49−7.24 (m, 10H), 7.44 (d, J = 1.0 Hz, 1H), 6.32 (dd, J = 9.0, 5.5 Hz, 1H), 6.02 (dd, J = 8.5, 5.5 Hz, 1H), 4.83 (m, 1H), 4.40 (m, 1H), 4.05 (m, 1H), 3.92 (m, 1H), 3.72 (m, 2H), 3.45 (m, 1H), 2.84, 1.04 (q, t, NEt_3), 2.07 (m, 2H), 1.92 (m, 1H), 1.80 (m, 1H), 1.76 (br), 1.71 (d, J = 1.0

Hz, 3H), 0.91 (s, 9H), 0.75 (s, 9H), −0.041 (ss, 6H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.36, 166.21, 152.44, 151.96, 138.03, 137.11, 136.86, 136.81, 134.36, 134.26, 131.19, 131.13, 129.07, 129.00, 112.13, 111.26, 88.11, 88.06, 88.04 (d, J = 1.7 Hz), 86.29, 88.24, 78.53 (d, J = 4.6 Hz), 76.20, 66.47 (d, J = 5.5 Hz), 64.90, 47.45 (NEt_3), 41.57, 40.43 (d, J = 4.6 Hz), 27.39, 26.51, 22.08, 19.74, 19.20, 12.72, 12.67, 9.68 (NEt_3), −5.08, −5.16.

Phosphorothioate (Rp-11) was obtained from Rp-**10** in 78% yield as described for Sp-**11**. Two diastereomers of Rp- and Sp-**11** were observed from its ^{31}P NMR in a ratio of around 24:1. ^{31}P NMR (202.3 MHz, CD_3OD): δ 58.92 ppm (Rp-**11**, 96%), 58.99 ppm (Sp-**11**, 4%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (d, J = 1.0 Hz, 1H), 7.80 (d, J = 1.0 Hz, 1H,), 6.29 (dd, J = 8.0, 6.0 Hz, 1H), 6.22 (dd, J = 8.2, 5.5 Hz, 1H), 5.00 (m, 1H), 4.45 (m, 1H), 4.15 (m, 1H), 4.06 (m, 2H), 3.98 (m, 1H), 3.77 (m, 2H), 3.17, 1.59, 1.35, 0.95 (NBu_4^+), 2.40 (m, 1H), 2.20 (m, 2H), 2.14 (m, 1H), 1.91 (d, J = 1.0 Hz, 3H), 1.81 (d, J = 1.0 Hz, 3H). ^{13}C NMR (125.7 MHz, CD_3OD): δ 166.43, 166.28, 152.39, 152.21, 138.12, 138.03, 112.00, 111.48, 87.85 (d, J = 5.5 Hz) 87.49, 87.42, 86.20, 86.08, 77.49 (d, J = 5.5 Hz), 72.94, 66.35 (d, J = 6.4 Hz), 62.83, 59.44 (t, NBu_4^+), 40.87, 39.88 (d, J = 4.5 Hz), 24.70 (NBu_4^+), 20.62 (NBu_4^+), 13.87 (NBu_4^+), 12.61, 12.40.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for all compounts in the Experimental Section and ^{31}P NMR spectra for compounds **7a**, **7b**, **9a**, **9b**, **10**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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