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A Novel Nucleoside Phosphoramidite Synthon Derived from 1*R*,2*S*-Ephedrine

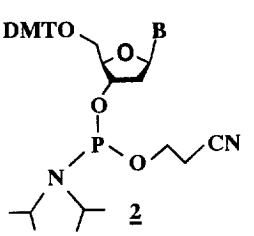
Radhakrishnan P. Iyer, Dong Yu, Nan-Hui Ho,[a] Weitian Tan and Sudhir Agrawal

Hybridon Inc., One Innovation Drive, Worcester, MA 01605.
[a]Department of Chemistry, Tufts University, Medford, MA 02155

Abstract: The stereoselective synthesis of the nucleoside phosphoramidite **6** by reaction of the chiral oxazaphospholidine **4** with 5'-DMT-T is reported. The novel phosphoramidite synthon, **6**, is stereoselectively converted to **8** by oxidative sulfurization with **1**. The oxazaphospholidines, **6** and **8**, are a new class of potential nucleoside synthons for the synthesis of oligonucleotides.

Oligonucleoside phosphorothioates (PS-oligos) have shown great therapeutic potential as antisense-mediated inhibitors of gene expression[1] as evidenced by a number of ongoing clinical trials against AIDS and cancer.[2,3] These PS-oligos, currently employed in clinical studies and biological evaluations, are obtained as mixtures of 2^n diastereomers (n = number of internucleotidic phosphorothioate linkages) during synthesis. To date only limited data is available on the comparative biophysical and biological properties of stereodefined phosphorothioates[4a,b] due to non-availability of sufficient quantities of completely "stereoregular" PS-oligos of sufficient length. Enzymatic synthesis[4c] gives only R_p-phosphorothioates and is not as yet amenable to large-scale work. Therefore, there exists a need to develop practical stereoselective synthesis of oligonucleoside phosphorothioates with defined *P*-stereochemistry.

The large-scale synthesis of oligonucleoside phosphorothioates is presently carried out by initial formation of the internucleotidic phosphite linkage using solid-phase phosphoramidite chemistry[5a,b] followed by oxidation of the phosphite to phosphorothiate using the thiolsulfonate reagent **1**.[6] For this purpose, nucleoside β-cyanoethyl phosphoramidite **2** is the most widely used monomer synthon. We propose to synthesize oligonucleoside phosphorothioates using the phosphoramidite nucleoside synthons, such as **3**, incorporating a variety of commercially available chiral auxiliaries. Our objective is to examine whether the chiral auxiliary can be used to effect diastereoselectivity in the formation of internucleotidic phosphorothioate linkage.



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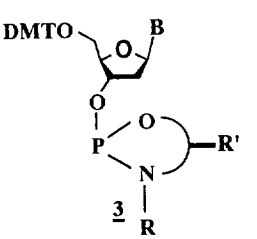

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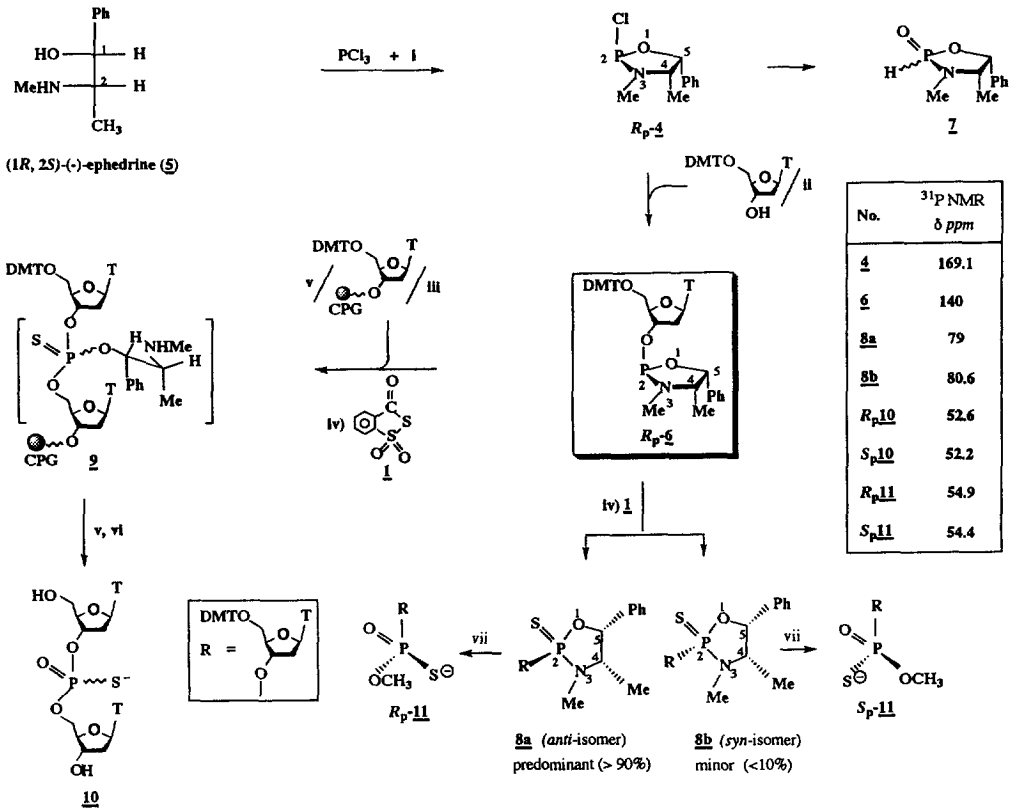
Recent reports[7a,b] regarding the synthesis of the chlorophosphoramidite **4** derived from (1*R*,2*S*)-ephedrine (**5**) and its use in stereoselective P-C, P-O and P-N bond forming reactions prompted us to examine the use of **4** in the synthesis of the nucleoside phosphoramidite synthon **6** and evaluate its potential in the development of a stereoselective synthesis of oligonucleoside phosphorothioates. Reported herein is the preliminary results of our studies on the synthesis of **6** and model studies on the synthesis of dinucleoside phosphorothioates **10** using **6**.



i) N-methyl morpholine, toluene, -78 °C, 3 h, then 22 °C, 12 h; 75% yield ii) ethyl ether, pyridine:$N(Et)_3$, 1:4, -78 °C, 3 h then 22 °C, 12 h; 84% yield iii) tetrazole (95% coupling efficiency) iv) **1** used as a solution in acetonitrile, 30 sec, 22 °C v) dichloroacetic acid (2% in methylene chloride) vi) Aq. NH_4OH (28%), 55 °C, 1 h. vii) NaOMe/MeOH, 0 °C, 3 h then 25 °C, 12 h; 28% NH_4OH, 55 °C, 4 h.

SCHEME 1

The chlorophosphoramidite, (2*R*,4*S*,5*R*)-2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine (**4**), (Scheme 1) was obtained by reaction of 1*R*,2*S*-ephedrine (**5**) with PCl_3 in the presence of N-methyl morpholine at -78 °C, as described,[7a,b] but with minor modifications. ^{31}P-NMR examination of the crude reaction mixture revealed the presence of a predominant isomer (> 95%) at δ 169.4 *ppm* and a minor component (< 5%) at δ 161 *ppm*. Upon vacuum distillation, a colorless liquid was obtained (95-97 °C at 0.1 mm Hg)[8a] which solidified upon cooling to -78 °C (isolated yields of 75%). The spectral features of this compound[8b] were in agreement with reported values[7a,b] and the structure R_p-**4** was assigned to it in which the chlorine atom is *trans* disposed relative to the C-Ph and C-Me substituents in the phospholidine ring. **4** could be stored as a solid in a desiccator at -5 °C for several days with no apparent decomposition (as evaluated by ^{31}P-NMR). Addition of water to **4** gave the H-phosphonate **7** as a mixture of diastereomers (R_p:S_p, 55:45, ^{31}P-NMR).

Reaction of the chlorophosphoramidite **4** with 5'-*O*-DMT thymidine in the presence of triethylamine gave **6** as a single diastereomerically pure isomer in isolated yields ca. 84%.[9a] Examination of ^{31}P-NMR of **6** (Fig. 1) revealed a signal at δ 140 *ppm* corresponding to a single P-epimer. In analogy with substitution reactions of **4**

involving carbon-, oxygen and nitrogen-based nucleophiles,[7a,b] which gave substitution products with overall retention of configuration, **6** can be formulated as having the structure (Scheme 1) with R_p configuration. This hitherto unreported nucleoside phosphoramidite **6** is a white solid and is stable when stored dry at 0 - 5 °C.[9b] Oxidative sulfurization of the phosphoramidite **6** with thiolsulfonate **1**[6] gave the thiophosphoramidates **8a**:**8b** (90:10, 81% yield) (isomer ratio based on ^{31}P-NMR, Fig. 1).[10a] The predominant isomer **8a** (easily separated from **8b** by flash chromatography) has been tentatively assigned the configuration as indicated (Scheme 1).[10b] The assignment of configurations for **8a,b** is based on the generally accepted notion that P(III) oxidations proceed with high stereoselectivity and with overall retention of configuration.[5b,11]

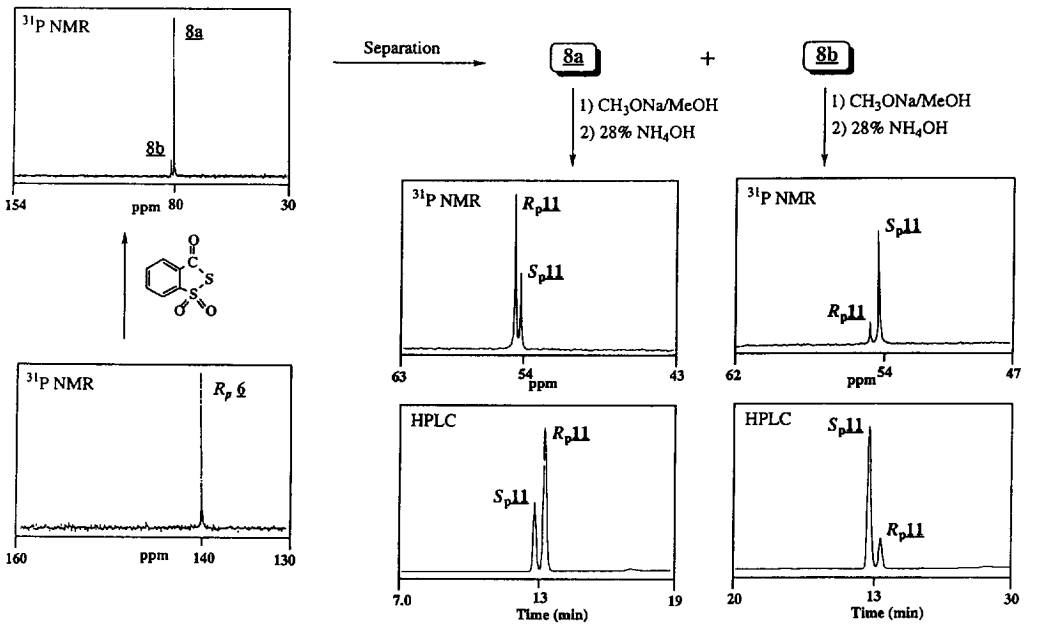


Fig. 1. ^{31}P-NMR and HPLC profiles of **6**, **8** and **11**.

Having obtained the nucleoside phosphoramidite **6** in preparative-scale reactions, the stage was set for its use in solid-phase coupling with CPG-bound nucleoside. Thus contacting a solution of **6** in acetonitrile with CPG-T(10 μmol) during a period of 30 minutes, in the presence of tetrazole, followed by oxidation with the thiolsulfonate **1** resulted in efficient formation of the product **9** in coupling efficiency greater than 95% (as evaluated by "trityl yields").[12a] Following synthesis, the CPG-bound product was heated with aqueous ammonium hydroxide (28%, 55 °C, 1h). Examination of the products by ^{31}P-NMR and reverse-phase HPLC[12b,c] revealed that the dinucleoside phosphorothioate **10** had been formed as a mixture of diastereomers (R_p:S_p, 40:60). Interestingly, the commonly used cyanoethylphosphate deprotection strategy (28% aq. NH_4OH, 55 °C) was found to be sufficient to cleave the chiral phosphate appendage in **9** and generate the phosphorothioate **10**. The integrity of the chiral auxiliary, following removal from **9**, has not however been fully established.[13a,b] The lack of high stereoselectivity in the formation of **10** is consistent with other reports wherein epimerization of the phosphorous center (in the case of stereoisomerically pure phosphoramidites) is observed when acidic type activators, e.g. tetrazole, are used, in conjunction with phosphoramidite methodology, in the synthesis of deoxyribonucleoside phosphorothioates.[4a,5b]

Our current efforts center around the use of the chiral thiophosphoramidate **8** (Scheme 1) in coupling with nucleosides in a sequence reminiscent of the phosphotriester methodology. We found that treatment of **8a** and **8b** with sodium methoxide in methanol at ambient temperature followed by heating with NH_4OH gave **11** in

90% yield and in moderate to high stereoselectivity (as monitored by ^{31}P-NMR and HPLC) (Fig. 1). The R_p:S_p ratio of **11** obtained from **8a** was 70:30 where as from **8b** was 10:90.[12c] These results suggest that good diastereofacial selectivity can be achieved in nucleoside coupling reactions using **8**.

In conclusion, we report here that oxazaphospholidines **6** and **8** are a new class of nucleoside synthons. Further studies are in progress to use the synthon **8** in the stereoselective synthesis of oligonucleotides.[14]

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8. (a) Reported[7b] b.p. 160 °C at 0.1 mm Hg. (b) **4**, ^{31}P-NMR ($CDCl_3$) (TMP ext. standard) δ *ppm* 169.1; ^{1}H-NMR ($CDCl_3$) δ *ppm* 0.71 (3H, d, J = 6.3 Hz), 2.69 (3H, d, $^3J_{P\text{-}H}$ = 15.1 Hz, N-CH_3), 3.63 (1H, ddq, J = 1.3, 5.5, $^3J_{P\text{-}H}$ = 7.6 Hz, H-4), 5.85 (1H, dd, J = 5.5 Hz, $^3J_{P\text{-}H}$ ~ 1.2 Hz), 7.15 (5H, m, -Ph).
9. (a) Yields are being optimized. (b) **6**, ^{31}P-NMR δ *ppm* 140.0; ^{1}H-NMR ($CDCl_3$) δ *ppm* 0.61 (3H, d, J = 6.5 Hz), 1.41 (3H, s, T-CH_3), 2.42 (2H, m, H-2'), 2.63 (3H, d, $^3J_{P\text{-}H}$ = 12 Hz, N-CH_3), 3.37 (1H, dd, J = 10.6, 2.6 Hz, H-5'), 3.46 (1H, dd, J = 10.6, 2.6 Hz, H-5'), 3.52 (1H, ddq, J = 6.9, 6.5 Hz, $^3J_{P\text{-}H}$ = 2.4 Hz, H-4), 3.76 (6H, s, -OCH_3), 4.08 (1H, m, H-4'), 4.91 (1H, m, H-3'), 5.56 (1H, dd, J = 6.9 Hz, $^3J_{P\text{-}H(5)}$ = 1.84 Hz, H-5), 6.41 (1H, dd, J = 6.7, 6.7 Hz, H-1'), 6.85 (4H, m, -Ph), 7.25 (14H, m, -Ph), 7.6 (1H, s, H-6), 9.1 (1H, s, -NH). FAB-MS (m/z) = 736 (M-H), $C_{41}H_{44}N_3O_8P$.
10. (a) **8a**, ^{31}P-NMR ($CDCl_3$) δ *ppm* 79.0; ^{1}H-NMR ($CDCl_3$) δ *ppm* 0.78 (3H, d, J = 6.6 Hz, -CH_3), 1.41 (3H, s, T-CH_3), 2.55 (2H, m, H-2'), 2.70 (3H, d, $^3J_{P\text{-}H}$ = 12.5 Hz, -NCH_3), 3.36 (1H, dd, J = 10.5, 2.3 Hz, H-5'), 3.56 (1H, dd, J = 10.5, 2.2 Hz, H-5') 3.76 (1H, ddq, J = 6.6, 6.1 Hz, $^3J_{P\text{-}H}$ = 12.3 Hz, H-4), 3.78 (6H, s, -OCH_3), 4.28 (1H, m, H-4'), 5.57 (1H, m, H-3'), 5.62 (1H, dd, J = 6.1 Hz, $^3J_{P\text{-}H(5)}$ = 2.8 Hz, H-5), 6.48 (1H, dd, J = 9.0, 5.6 Hz, H-1'), 6.85 (4H, m, -Ph), 7.26 (14H, m, -Ph), 7.62 (1H, s, H-6) 8.90 (1H, s, -NH). FAB-MS (m/z) = 769, $C_{41}H_{44}N_3O_8PS$. (b) The criterion of smaller coupling constant $^3J_{P\text{-}H(5)}$ for the *syn* compared to the *anti* isomer has been used to assign configurations of a series of oxazaphospholidine-2-ones.[7a,b,10c] We observed $^3J_{P\text{-}H(5)}$ of 2.8 and 4.2 Hz for **8a** (*anti*) and **8b** (*syn*) respectively. However unambiguous configurational assignments for **8a, b** must await determination by X-ray crystallography. (c) Schwalbe, C. H.; Chopra, G.; Freeman, S.; Brown, J. M.; Carey, J. V. *J. Chem. Soc. Perkin Trans.* 2, **1991**, 2081-90.
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12. (a) Coupling efficiency and yields are being optimized. (b) Iyer, R. P.; Yu, D.; Agrawal, S. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2471-76. (c) Configurations were assigned using the criteria which were reported for dinucleoside phosphorothioates.[12b]
13. (a) Cooper et al.,[13b] have reported the isolation of (-)-*trans*-1,2-dimethyl-3-phenylaziridine upon base-mediated hydrolysis of 2-alkoxy-3,4-dimethyl 5-phenyl-1,3,2-oxazaphospholidine 2-ones. (b) Cooper, D. B.; Hall, C. R.; Harrison, J. M.; Inch, T. D. *J. Chem. Soc. Perkin Trans. I*, **1977**, 1969-80.
14. RPI dedicates this paper to Dr. Semmangudi Sreenivasa Iyer.

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