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STEREOCHEMICAL STUDIES OF THE FORMATION OF CHIRAL INTERNUCLEOTIDE LINKAGES BY PHOSPHORAMIDITE COUPLING IN THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES

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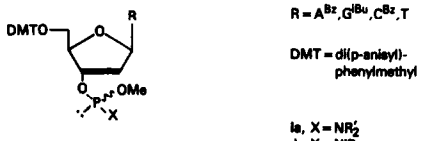
Abstract: HPLC-fractionated (R_p)- and (S_p)-phosphoramidites underwent tetrazole-catalyzed coupling to support-bound nucleosides to give intermediary phosphites with epimerization at phosphorus, as shown by conversion to epimerized mixtures of dinucleoside phosphorothioates.

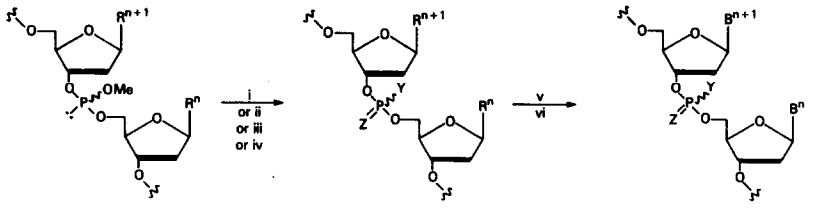
The use of deoxynucleoside phosphoramidites[2,3a] (Ia) for the solid-phase synthesis of either single-[2d,3] or mixed-sequence[4] oligonucleotides has met with considerable success. The phosphoramidite method[3a] is also exceptionally versatile in that dinucleoside O-methyl phosphite intermediates (II), which are normally oxidized to phosphates (IIIa), can instead be used to obtain, for example, IIIb-d, and, hence, oligonucleotide analogues IVb,[5-7] c,[6,8,9] or d,[10] respectively, having one or more[6a] backbone-modifications anywhere in the chain. Each of these modifications introduces an asymmetrically substituted internucleotide



R = A^{Bz}, G^{iBu}, C^{Bz}, T

DMT = di(p-anisyl)-phenylmethyl

Ia, X = NR'_2
b, X = $NiPr_2$
c, X = $NiPr_2$, R = C^{Bz}
d, X = tetrazoyl



II

IIIa, Y = OMe, Z = O
b, Y = OMe, Z = ^{17}O or ^{18}O
c, Y = OMe, Z = S or Se
d, Y = Ar_3C, Z = O

IVa, Y^- = Z = O
b, Y^- = O, Z = ^{17}O or ^{18}O
c, Y^- = O, Z = S or Se
d, Y = Ar_3C, Z = O

(i) H_2O-I_2-lutidine, (ii) [^{17}O]- or [^{18}O]-H_2O-I_2-lutidine, (iii) S_8-lutidine or KSeCN-CH_3CN, (iv) Ar_3CCl-CH_3CN, (v) PhSH-Et_3N, (vi) conc. NH_4OH-heat

linkage and, hence, the possibility of forming diastereomeric product mixtures, the composition of which being dependent upon the particulars of the reactions. Our automated synthesis of IVb-d[6,10] generally afforded roughly equimolar mixtures of diastereomeric products that were in some cases impossible to separate, and it was therefore of interest to investigate the mechanism of phosphoramidite coupling with the hope of ultimately maintaining the stereochemical integrity at phosphorus during the incorporation of I into II. Our initial findings regarding this problem are described herein.

Syntheses of I are known[2a-c] to afford a roughly equimolar mixture of the ($\underline{R}_p$) and ($\underline{S}_p$) diastereomers. The mixture of ($\underline{R}_p$)- and ($\underline{S}_p$)-I is then coupled to a support-bound 5'-HO acceptor moiety using tetrazole as a catalyst; typically, molar ratios of I : tetrazole : acceptor $\cong$ 10:50 : 1-0.5.[3] When this stoichiometry was employed with Ib, and the resultant intermediate was sulfurized with S_8-lutidine, products IVc were found to have roughly equal amounts of the ($\underline{R}_p$) and ($\underline{S}_p$) configurations, regardless of the nature of the flanking bases and the value of $\underline{n}$.[6] To distinguish between kinetically competitive but completely stereoselective coupling pathways for ($\underline{R}_p$)- and ($\underline{S}_p$)-Ib, as opposed to conceivable epimerization pathways, we decided to first examine the behavior of diastereomerically enriched samples of phosphoramidite. Elution of ~50:50 mixtures of ($\underline{R}_p$)-and ($\underline{S}_p$)-Ib from a C_{18} HPLC column (7.8mm x 30cm, μBondapak®, Waters Assoc.) using CH_3CN ($<$0.01% H_2O, 2.5 mL/min) led to partial resolution of the diastereomers of Ib with R=C^{Bz} (Ic, 8.24 and 8.70 min), but not with R=A^{Bz} (6.27 min), G^{iBu} (8.04 min), or T (6.26 min). In this manner we obtained fractions of the faster-eluting (Ic-Fast) and slower eluting (Ic-Slow) diastereomers having >95:5 and 25:75 molar ratios of Ic-Fast:Ic-Slow, as determined from ^{31}P NMR spectra recorded in CH_3CN (Ic-Fast, 140.19 ppm and Ic-Slow, 139.89 ppm, relative to external H_3PO_4 in D_2O). These fractions, on a batch-to-batch basis, contained variable amounts (5-20%) of diastereomeric nucleoside 3'-$\underline{O}$-methyl phosphonates (~0 ppm, $^1J_{PH} \cong$ 715 Hz), which were attributed to adventitious hydrolysis of the P-N bond, and could be produced by addition of tetrazole to an NMR sample of Ic in CH_3CN that contained a trace amount of water. The configurational stability at phosphorus in Ic with regard to possible reversible reactions with either the support matrix, tetrazole, or $HNiPr_2$, which is released during coupling, was demonstrated by ^{31}P NMR control experiments (not reported). The expected[11] stereoretentive nature of the reaction of intermediate II with S_8-lutidine was supported by ^{31}P NMR monitoring of the completely stereoselective conversion of Ic-Fast and Ic-Slow into their corresponding P=S derivatives (64.04 ppm and 64.76 ppm, respectively).

The enriched samples of Ic-Fast and Ic-Slow, as well as unfractionated Ic, were separately coupled, in parallel syntheses, to support[2d]-bound dC (1 μmol) using the following conditions: (a) 10:50:1 molar ratios of Ic:tetrazole:dC-acceptor, (b) mixing of Ic and tetrazole during delivery (20s) to the support, and (c) 3 min for reaction at room temperature (R.T.). Sulfurization of the resultant II with 0.4M S_8-lutidine (30 min, 60°C) followed by sequential detritylation (3% Cl_3CCO_2H-CH_2Cl_2, 100s, R.T.), $\underline{O}$-demethylation (1:1 PhSH-Et_3N, 30 min, R.T.),

cleavage from the support (NH_4OH, 2 h, R.T.), base-deprotection (NH_4OH, 10 h, 60°C), and concentration *in vacuo* gave samples of crude d($C_{PS}C$). HPLC analyses[6] using previously synthesized[6] standards revealed that the 3 samples of the crude product contained a 55:45 mixture of (R_p)- and (S_p)-d($C_{PS}C$), as well as some d(CC) (4%) and dC (3%) due to incomplete sulfurization and incomplete coupling, respectively. Parallel coupling reactions of diastereomerically enriched Ic-Slow and unfractionated Ic with support-bound dG likewise gave identical 56:44 mixtures of diastereomeric (R_p)- and (S_p)-d($C_{PS}G$), together with d(CG) (8%) and dG (4%). Additional data (not reported) established that the phosphite moiety in II is not epimerized by tetrazole, and that the the phosphorothioate linkages are configurationally stable under the conditions used for detritylation, *O*-demethylation, and base-deprotection. Taken together, the foregoing results were consistent with epimerization at phosphorus prior to coupling, which was further investigated as described below.

Tetrazoylamidites (Id) have been discussed[12] as candidates for the reactive species during tetrazole-catalyzed coupling of Ia, and it seemed reasonable to us that Id could be formed completely stereoselectively but then undergo epimerization at phosphorus by chemically degenerate reactions with tetrazole [*i.e.*, (R_p)-Id + tetrazole* = (S_p)-Id* + tetrazole] on a time-scale that was fast, relative to coupling. To determine if substantially decreasing the relative concentration of tetrazole could be used to kinetically disfavor this hypothetical epimerization process but still afford good coupling efficiency, the synthesis of d(CG) was performed using 10- and 100-fold reductions in the amount of tetrazole that was normally co-delivered with Ic to the support, which gave only 55% and 2% yields of the dimer, respectively, even with prolonged times for reaction. Replacement of tetrazole (pK_a 4.8) with either octanoic acid (pK_a 4.9) or Et_3N as potential alternative catalysts gave 0.5% and <0.3% yields of d(CG), respectively. These discouraging results prompted a ^{31}P NMR spectroscopic study of an optimized, solution-phase, test-case, *viz.*, separate reactions of the enriched samples of Ic-Fast and Ic-Slow with a 1,300-fold molar excess of absolute EtOH (as solvent) in the presence of only 10 mol-% tetrazole, relative to Ic. Spectra obtained 15 min after the addition of tetrazole showed that, in both cases, the nucleoside 3'-*O*-methyl-*O*-ethyl phosphite diastereomers (9.58 and 9.66 ppm upfield from Ic-Slow) were formed at roughly equal rates, and thus revealed that the rate constant for epimerization at phosphorus was orders of magnitude greater than the rate constant for coupling.[13] Inasmuch as the relative concentration and effective nucleophilicity of a support-bound 5'-HO-nucleotide moiety are far less than that of the EtOH nucleophile, it was concluded that alternative catalysts[12] for coupling of Ia, or other phosphitylating reagents, need to be investigated to achieve a versatile, stereochemically defined synthesis of P-chiral analogues of DNA.

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(13) Although mechanistic differences are operative, it should be mentioned that reactions of alcohols with optically active ethyl phenyl phosphinous acid diethylamide proceed with complete racemization [L. Horner and M. Jordan, Phosphorus and Sulfur, 8, 235-242 (1980)]. Also noteworthy is that the presently reported data do not allow us to distinguish between tetrazole-mediated epimerization of Id and either rapid inversion at phosphorus [cf. G. Zon, W. Egan, R. Tang, and K. Mislow, J. Am. Chem. Soc., 93, 6205-6216 (1971)] or local planarity at phosphorus.

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