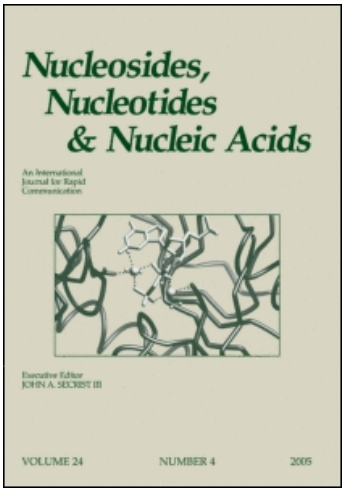

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Michal Sobkowski[a]; Jacek Stawinski[ab]; Adam Kraszewski[a]
[a] Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland [b] Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

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A PROPOSAL FOR A CONVENIENT NOTATION FOR P-CHIRAL NUCLEOTIDE ANALOGUES. PART 4. A RELATIONSHIP BETWEEN THE D_P/L_P NOTATION AND STEREOCHEMISTRY OF REACTIONS

Michal Sobkowski,[1] Jacek Stawinski,[1,2] and Adam Kraszewski[1]

[1]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland*
[2]*Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden*

□ *Recently, we have proposed a new D_P/L_P stereochemical notation for* P*-chiral dinucleoside monophosphate analogues based on a structural relationship between compounds. As an extension of this work, we present here applications of the D_P/L_P notation for tracking stereochemistry of reaction pathways involving H-phosphonate, phosphoramidite, phosphorotriester, and other intermediates frequently met in the nucleotide chemistry.*

Keywords Stereochemical notation; D_P/L_P; P-chiral compounds; nucleotide analogues

INTRODUCTION

In the previous parts of this series, we presented a concept of a new notation for configuration of P-chiral compounds containing nucleosides or their analogues.[1–3] According to this proposal, four ligands at the phosphorus center of a nucleotide derivative are designated as $\mathbf{G_1}$, $\mathbf{G_2}$, **X**, and **Z**, and the compound is presented in a Fischer-like projection, in such a way that ligands $\mathbf{G_1}$ and $\mathbf{G_2}$ occupy vertical, and **X** and **Z**, horizontal positions, as shown in Figure 1. The positions of ligands $\mathbf{G_1}$ and $\mathbf{G_2}$ are fixed ($\mathbf{G_1}$ is an upper unit and $\mathbf{G_2}$, the lower unit), and the D_P/L_P configuration of a *P*-chiral center is determined by a relative position of ligands **Z** and **X**.

D_P configuration is defined as one having a single-bonded ligand **Z** to the right, and the phosphoryl group (or its analogue) to the left. For L_P

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Address correspondence to Michal Sobkowski, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland. E-mail: msob@ibch.poznan.pl

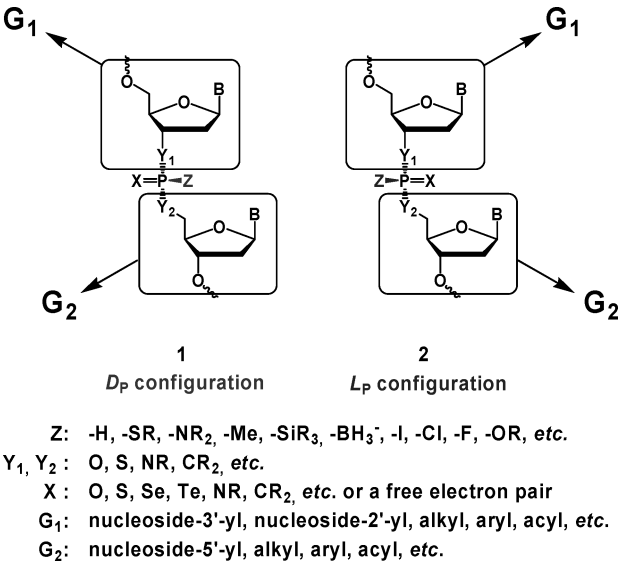

FIGURE 1 Structures for definition of the extended D_P/L_P system.

configuration, the arrangement of ligands **Z** and the phosphoryl function are opposite, that is, **Z** group in place to the left, and the P=X group, to the right.

In this article, various aspects of application of the D_P/L_P convention to denote stereochemical courses of the reactions involving P-chiral phosphorus intermediates are discussed.

BASIC GUIDELINES

The D_P/L_P notation is a simple and informative nomenclature system for specifying sense of chirality of phosphorus compounds, particularly useful when analyzing properties of P-chiral nucleotide analogues. Apart from this, the new system seems to be also more convenient than the R_P/S_P notation for analysis of stereochemical courses of reactions involving phosphorus center as in a majority of cases the inversion of configuration is clearly reflected in changes of the stereochemical descriptors from D_P to L_P (or vice versa), while for the reactions occurring with retention of configuration, the D_P/L_P descriptors remain unchanged (Figure 2).

Besides these typical reactions, however, we have identified instances in which D_P/L_P descriptors cannot be immediately assigned as, due to the adopted D_P/L_P rules, the produced compounds or intermediates would have ligands in incorrect positions, or are mesomeric anions with incorrect designation of a double bond (P=X). These cases are discussed in detail below.

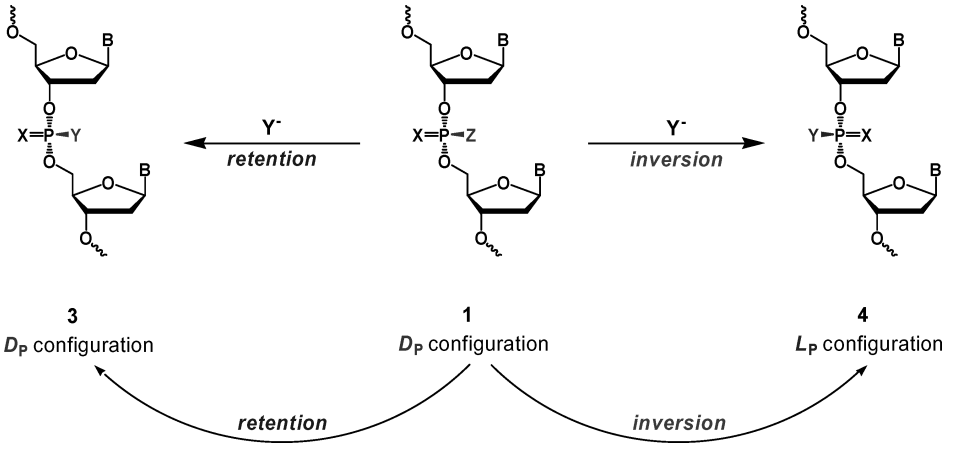

FIGURE 2 Typical relations between stereochemistry and its notation in the D_P/L_P system. The example shows transformations of D_P isomer (**1**); L_P isomer (**2**) yields **4** (L_P, retention) or **3** (D_P, inversion), analogously.

Reactions Yielding Structures Violating the D_P/L_P rules—D_P*/L_P* Notation

In an exemplary reaction shown in Figure 3, D_P-(R_P) phosphate triester **7** is formed by oxidative coupling of D_P-(S_P) ethyl nucleoside *H*-phosphonate **5** with another nucleoside. In the first stereoretentive step of this process, the resulting phosphoroiodidate **6** has incorrect graphical presentation for the assignment of D_P/L_P descriptors,* although positions of ligand **Z** (an iodine) and **X** (an oxygen) in a Z–P=X moiety are as in compounds with D_P configuration. A similar situation happened for triester **7b** in which a newly added nucleoside moiety occupies **Z** position, while it should be a $\mathbf{G_2}$ ligand in order to assign D_P/L_P descriptors to this compound.

In such instances as these, we propose to use D_P/L_P stereochemical descriptors tagged with an asterisk, D_P^*/L_P^*, to denote a violation of the D_P/L_P notation rules for a given structure having, however, a correct spatial arrangement of ligands (absolute configuration). By adopting this simple convention, the D_P/L_P and D_P^*/L_P^* stereochemical descriptors will always reflect a stereochemical outcome of the reaction. For the sake of simplicity of the descriptor system, we recommend to use D_P^*/L_P^* in the place

*According to Rule 4A of the D_P/L_P notation, a ligand of lower CIP priority (ethoxy in this case) should be assigned as **Z** and the one of higher CIP priority (iodine) as $\mathbf{G_2}$.

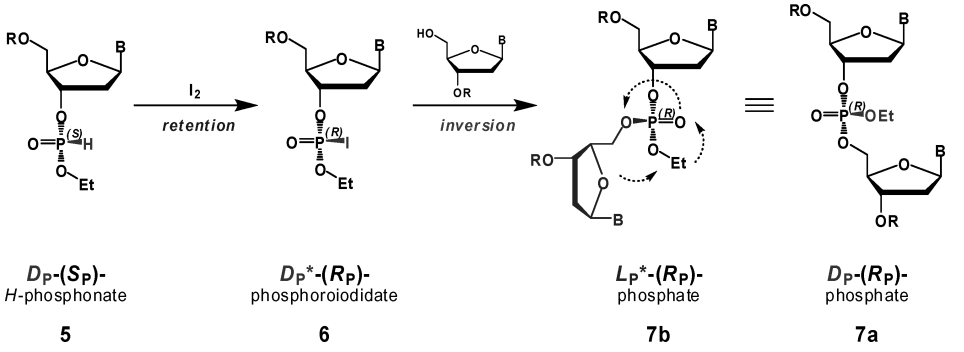

FIGURE 3 Stereochemistry of oxidative coupling of ethyl nucleosidyl *H*-phosphonate with nucleoside.

of *pseudo-D*P*/pseudo-L*P notation (Figure 4)[†] for tracking a stereochemical course of the reaction.

Phosphotriester **7b** with L_P*-(R_P) descriptor can be converted into a proper graphical presentation for D_P/L_P notation (structure **7a**) by two consecutive replacements of the ligands as in typical manipulations of the Fischer projection (these are equivalent to an imaginary rotation of the phosphate moiety, taking P-$O_{nucleoside}$ bond as a pivot) as shown in Figure 3.

Although the conversion of L_P* into D_P or D_P* into L_P can be done for any intermediate structure, however, since this operation involves a formal change of the descriptor, it is advisably to do it for a final product of the reaction, for example, to compare its stereochemistry with a compound produced in a different reaction pathway.

An example of reactions involving ambident anionic diesters is shown in Figure 5. In the reaction sequence **(a)**, D_P-(S_P)-*H*-phosphonate **10** is sulfurized to give phosphorothioate **8a**, which upon alkylation yields *S*-alkyl phosphorothioate **11**. The replacement of the P-H bond in **10** by sulfur (retention of configuration) produces phosphorothioate **8a** in a mesomeric form (O=P–S^-) which is a legitimate form for assigning D_P configuration to this compound [**8a** D_P-(R_P)]. For this and the subsequent reaction, the D_P/L_P descriptors indicate retention of configuration at each step (**10**-D_P → **8a**-D_P → **11**-D_P).

[†]In the case of ambident phosphate anions, for example, dinucleoside phosphorothioates (Figure 4), two mesomeric forms should be considered. Due to the D_P/L_P rules for the assignment of **Z** and **X** ligands (a priority order of elements for double bond to phosphorus: P=O > P=S > P=Se > P=Te > P=N) only one type of mesomer has a legitimate structure for the assignment of D_P/L_P configuration [diester **8a**-D_P-(R_P) and **9a**-L_P-(S_P) with an O = P-S^- bonding system]. The other mesomeric form with a S=P–O^- bonding pattern has single and double horizontal bonds as in L_P configuration, but since it has oxygen as ligand **Z** (instead of sulfur as required by the D_P/L_P rules), prefix "*pseudo*" is used to distinguish it from structures with a proper L_P configuration [e.g., **8b**-*pseudo*-L_P-(R_P) or **9b**-*pseudo*-D_P-(S_P)]. In this way both mesomeric forms can be clearly discriminated and conveniently used in writing reaction schemes.

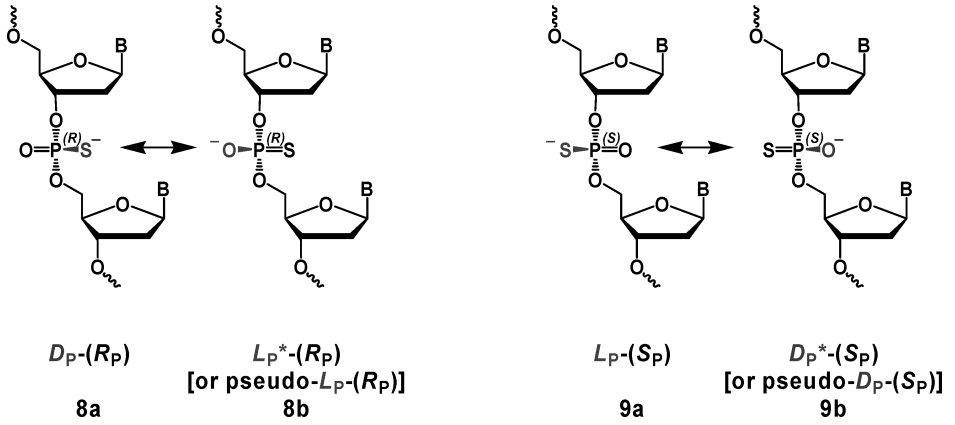

FIGURE 4 Mesomeric forms of D_P-(R_P) phosphorothioate anion and its L_P-(S_P) epimer.

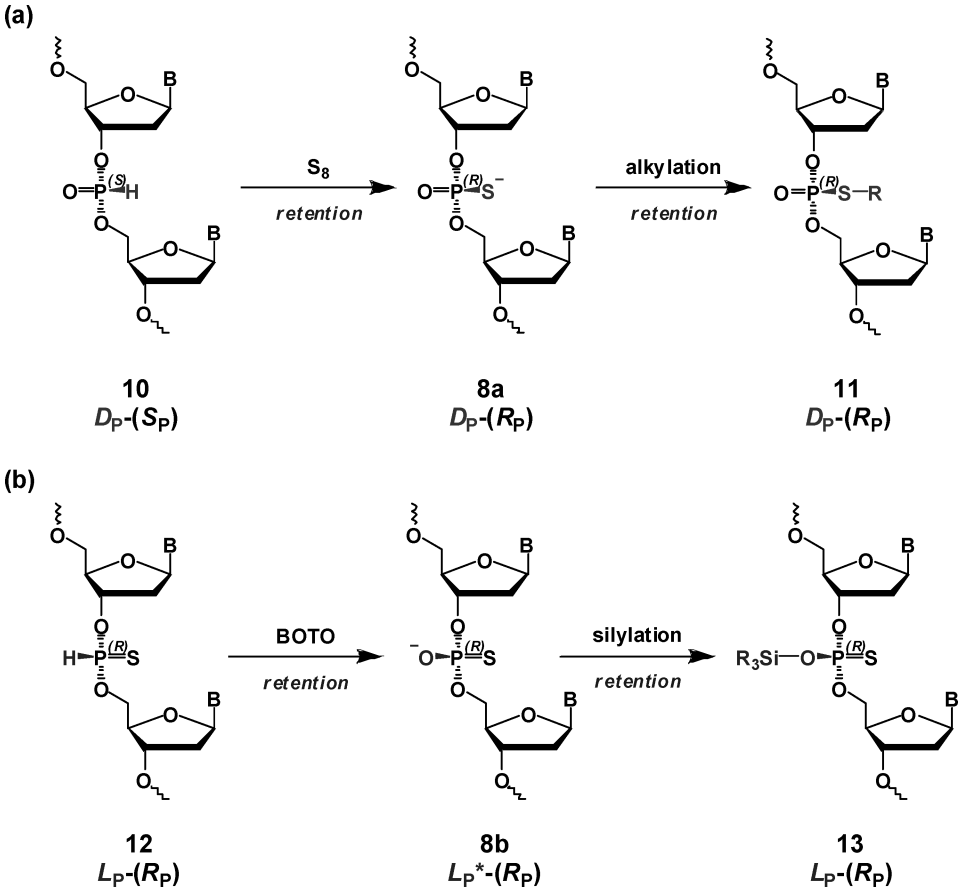

FIGURE 5 Reaction pathways involving ambident mesomeric phosphorothioate diesters.

In the sequence **(b)** in Figure 5, both reaction steps are stereoretentive as well. However, in the first reaction[4] replacement of the P-H bond by the P-O$^-$ one produces compound **8b** with apparent L_P configuration but in a *pseudo* bonding pattern ($^-$O–P=S instead of O=P–S$^-$). For this reason the stereochemical descriptor for **8b** is L_P*. Since the staring material, *H*-phosphonothioate **12** had L_P-(R_P) configuration, the stereochemical description of the reaction with the D_P/L_P convention indicates a retention of configuration (**12**-L_P → **8b**-L_P*). Upon silylation of **8b** the produced silylated phosphorothioate **13** has all the ligands in proper positions and its configuration can be directly read out from the structure [**13**-L_P-(R_P)]. Again, the D_P/L_P descriptors indicate retention of configuration (**8b**-L_P* → **13**-L_P).

DISCUSSION

Examples of Chemical Transformations of *H*-Phosphonate Derivatives

An application of D_P/L_P descriptors for selected transformations involving *H*-phosphonate intermediates is shown in Figure 6.

H-Phosphonate monoesters (e.g., **14**) are prochiral at the phosphorus center; however, after activation, the phosphorus atom becomes chiral and the further steps may be stereochemically defined. Esterification of L_P-(S_P) diastereomer of mixed anhydride **15** with nucleoside yields *H*-phosphonate diester **10**-D_P (inversion at the phosphorus[‡]). This compound can be stereoretentively converted into phosphorothioate **8a**-D_P, boranophosphate **17**-D_P or phosphoroiodidate **16**-D_P. The last compound, upon treatment with amine yields phosphoramidate **18**-L_P (inversion). In contrast to the CIP convention, in all these reactions the D_P/L_P descriptors correctly follow the inversion/retention stereochemistry at the phosphorus center.

Examples of oxidative coupling of *H*-phosphonate diester **10**-D_P and its thio analogue **18**-D_P with an alcohol are shown in Figure 7. In these cases, the D_P/L_P notation reflects the stereochemical course of the reactions correctly while the R_P/S_P descriptors change randomly (similar situation is observed if other nucleophiles, e.g., amines, are oxidatively coupled with dinucleoside *H*-phosphonates).

[‡]Stereochemistry of reactions proceeding with inversion of ligands placed vertically is reflected by exchanging positions of horizontal ligands, leaving molecules in the Fischer-like presentation. Such an approach allows immediate read out of the D_P/L_P notation.

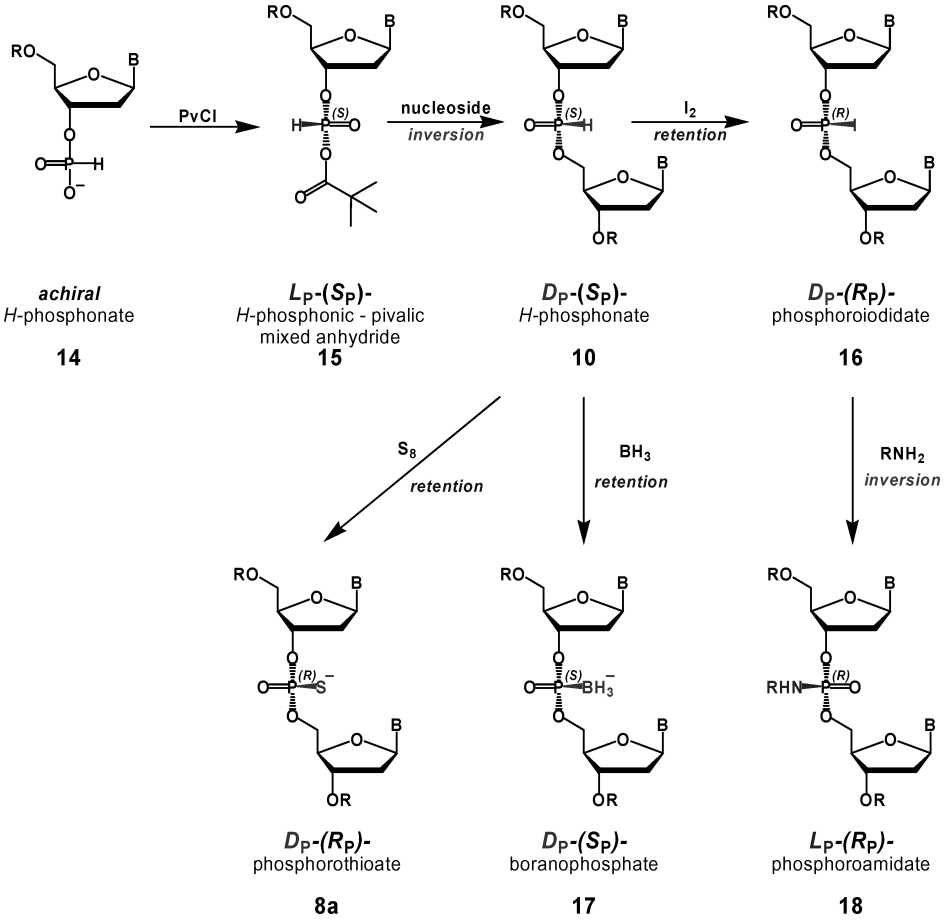

FIGURE 6 Examples of stereochemistry in chemical transformations of the *H*-phosphonate derivatives.

Examples of Chemical Transformations of Phosphoramidite Derivatives

In the phosphoramidite approach to the oligonucleotide synthesis the starting reactants are the respective nucleoside phosphoramidites used as a mixture of *P*-diastereoisomers. Although the separation of nucleoside phosphoramidite *P*-epimers is relatively simple, however, these undergo rapid epimerization at the phosphorus centre during activation of phosphoramidite synthons[6] as it is shown on the example of tetrazole as an activating agent (Figure 8). Nevertheless, it is possible to analyze the particular stereochemical tracks of presented reactions, applying the proposed herein extended D_P/L_P notation to show its logical simplicity and consequence.

It should be noted that for phosphoramidite synthons, the D_P/L_P convention directs the amide moiety into the horizontal position (as in **23a**). This moiety is subsequently substituted by a nucleosidic one, leading

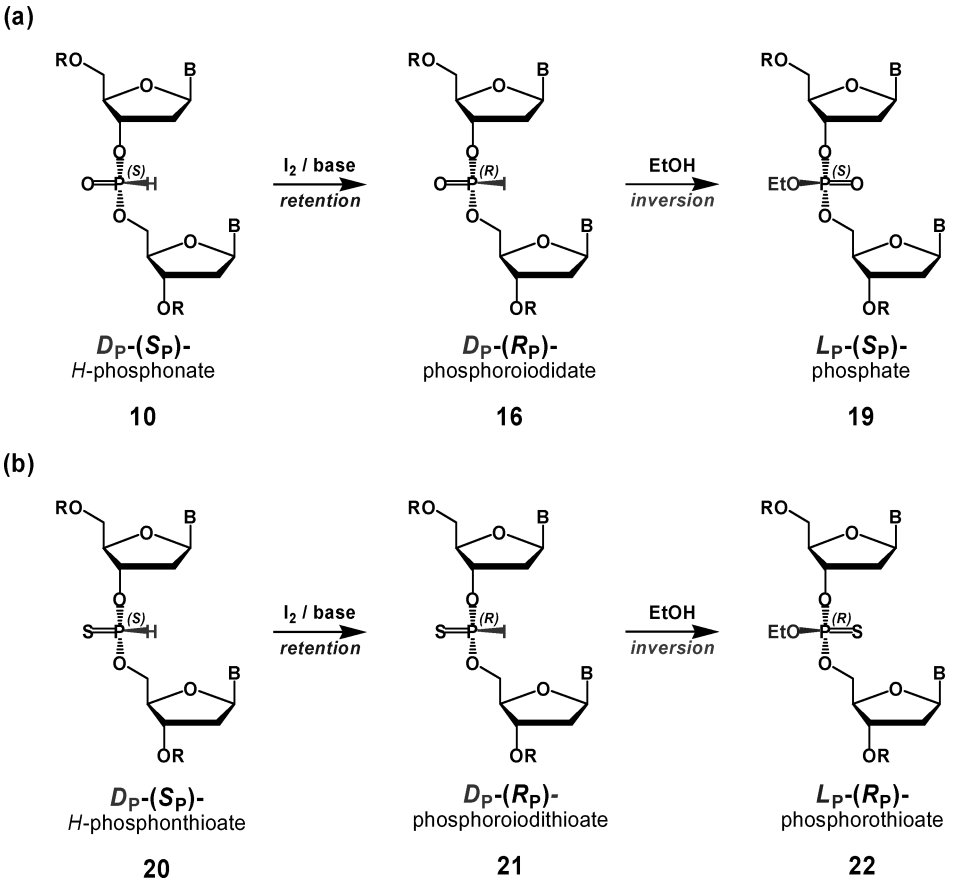

FIGURE 7 Stereochemistry of oxidative coupling of (a) *H*-phosphonate and (b) *H*-phosphonothioate diesters with ethanol (adapted from Stawinski et al.[5]).

to structures similar to triester **7b** (Figure 3). Such illegitimate D_P/L_P presentations of a compound can be used successfully for further analysis, however, in our opinion it is more convenient to change the presentation of the initial L_P phosphoramidite into its D_P* form having an amide moiety in the vertical position (structure **23b**, Figure 8). This structure can be used as a precursor for dinucleoside esters which will direct the next nucleosidic unit into the correct vertical position.

Thus, the stereochemistry of multistep transformation of **23b** into dinucleoside phosphorothioate **9b**[§] (Path A, Figure 8) consisting of several inversions and retentions of configuration can be easily decoded by tracking the descriptors of substrates and products where inversions change L_P to

[§]The final phosphorothioate diester **9b** in "Path A" is shown as in a phosphorothionate mesomer and thus its stereochemical descriptor is D_P* ("*pseudo*" S=P–O$^-$ bond arrangement). If there is a need, for instance, for comparison with a reference compound, D_P/L_P-correct phosphorothiolate mesomer **9a** with the $^-$S–P=O bonding pattern and L_P configuration sohould be used (Figure 8).

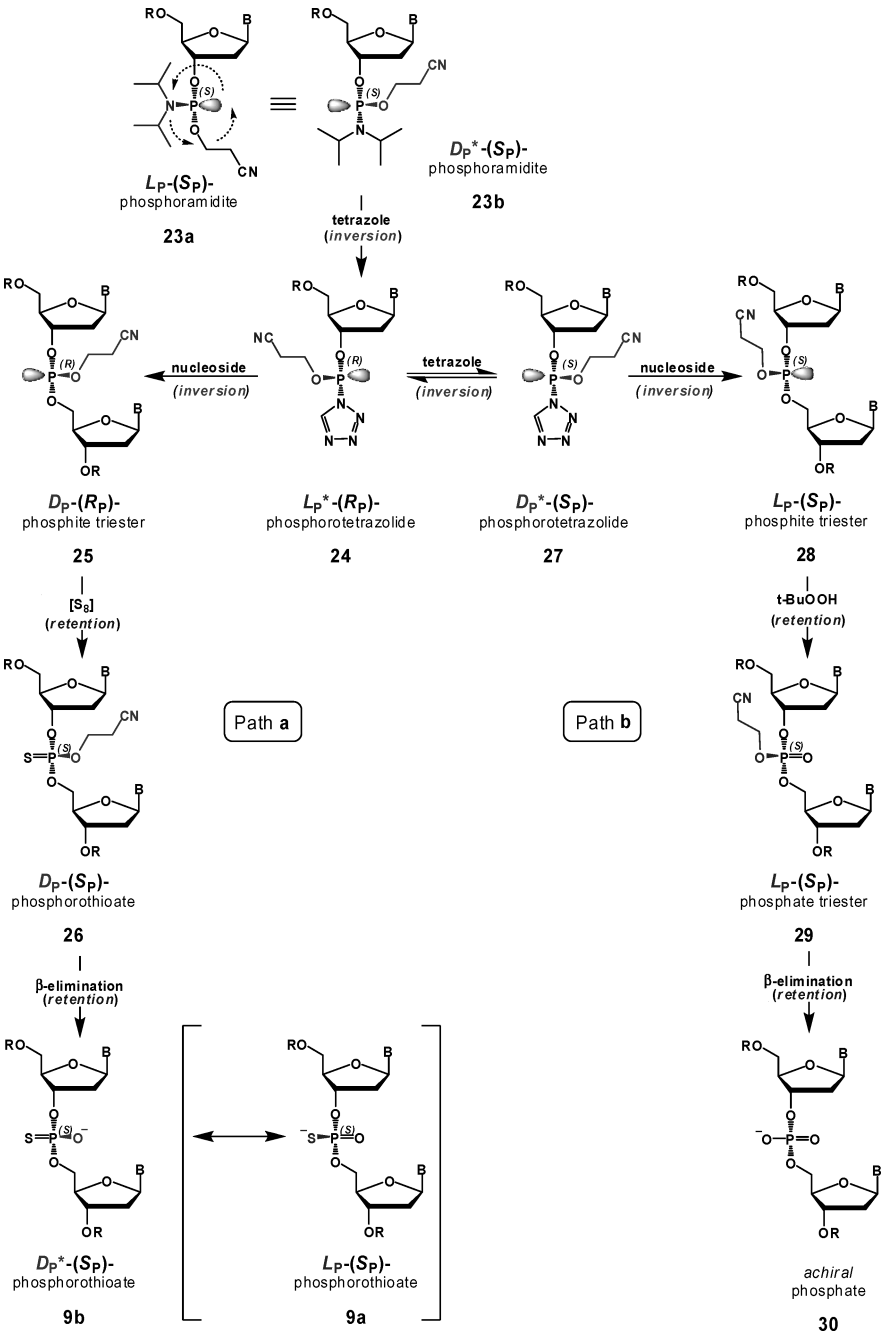

FIGURE 8 An example of stereochemistry in the phosphoramidite approach.

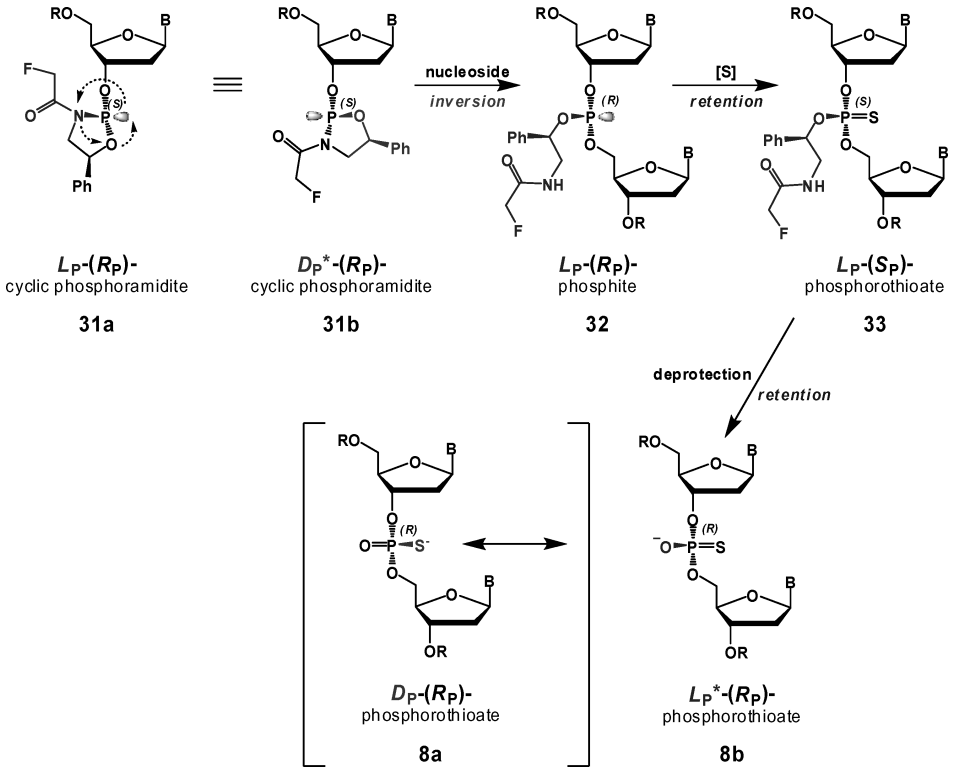

FIGURE 9 Stereocontrolled synthesis of dinucleoside phosphorothioate.

D_P (or D_P to L_P) while retentions keep descriptors unchanged (without or with the asterisk). The same is valid for the second presented reaction path (**23b** to **30**, Path B), which after several chiral transformations accurately described using the D_P/L_P convention ends up with achiral dinucleoside phosphate **30**.

Another example, dealing with stereocontrolled synthesis of phosphorothioates from a cyclic phosphoramidite **31** is shown in Figure 9.[7] After initial rotation of the cyclic substrate which puts the leaving group in a vertical $\mathbf{G_2}$ position (D_P* structure **31b**), the D_P/L_P notation tracks correctly the stereochemistry of each step involved throughout the reaction sequence to the L_P* phosphorothioate **8b**. For referencing purposes, **8a**-D_P mesomeric form of the product (shown in brackets) should be used.

Examples of Chemical Transformations of Phosphotriester Derivatives

Preparation of dinucleoside phosphorothioates by the triester approach can be achieved using either *S*-protected[8] or *O*-protected[8–10] substrate. In the first case (Figure 10), the prochiral phosphorothiolate **34** is

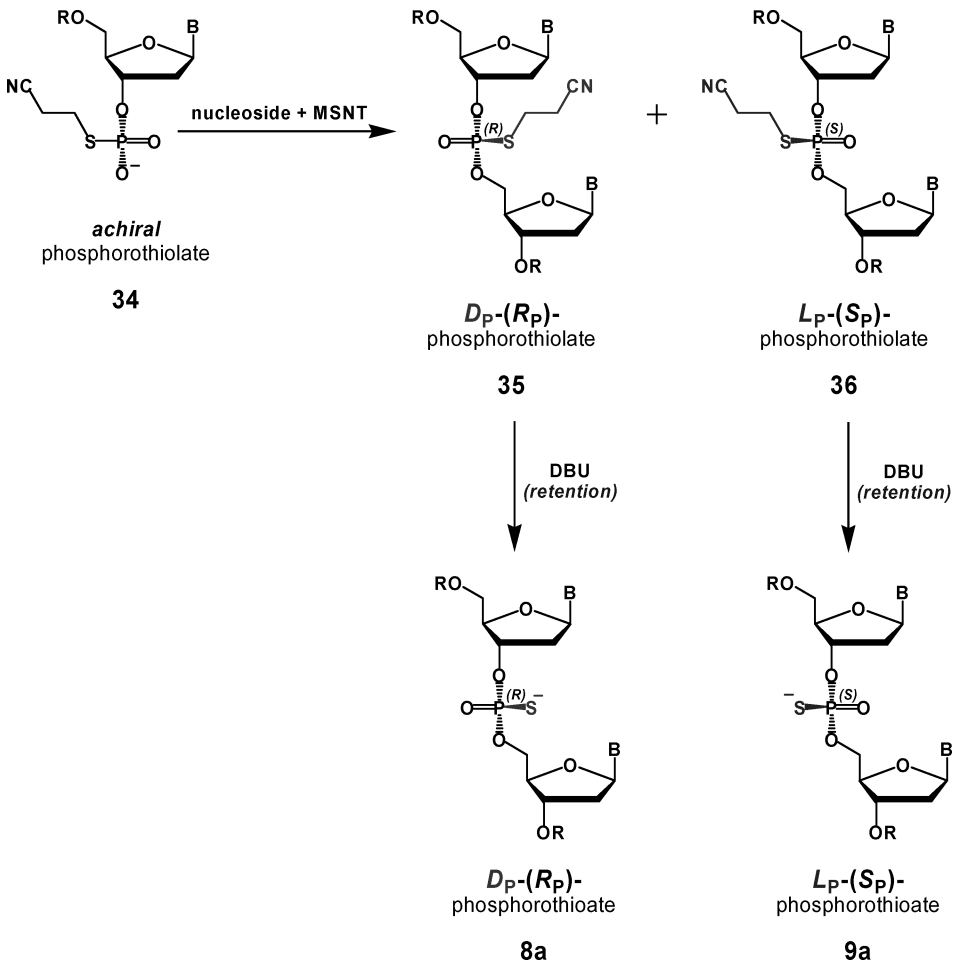

FIGURE 10 Triester approach to dinucleoside phosphorothioates (S-protection).

condensed with a nucleoside yielding a mixture of diastereomers of the *S*-protected triesters **35** (D_P) and **36** (L_P). These can be stereospecifically deprotected to phosphorothioate diesters **8a** (D_P) and **9a** (L_P), respectively. The D_P/L_P descriptors reflect correctly the actual stereochemistry of both reactions. Accidentally, in this instance also the R_P/S_P notation follows the stereochemical course of the reactions.

The second triester strategy for the synthesis of dinucleoside phosphorothioates requires *O*-aryl esters of type **37**, as a starting material (Figure 11). While the necessity of using nucleophilic catalysts (*e.g. N*-methylimidazole) for efficient condensation usually prevents any stereospecificity of such reactions, it is still possible to discuss the stereochemistry separately for each diastereomer. The first step of the reaction involves oxygen atom of phosphorothioate moiety as a nucleophile, so the mesomeric form

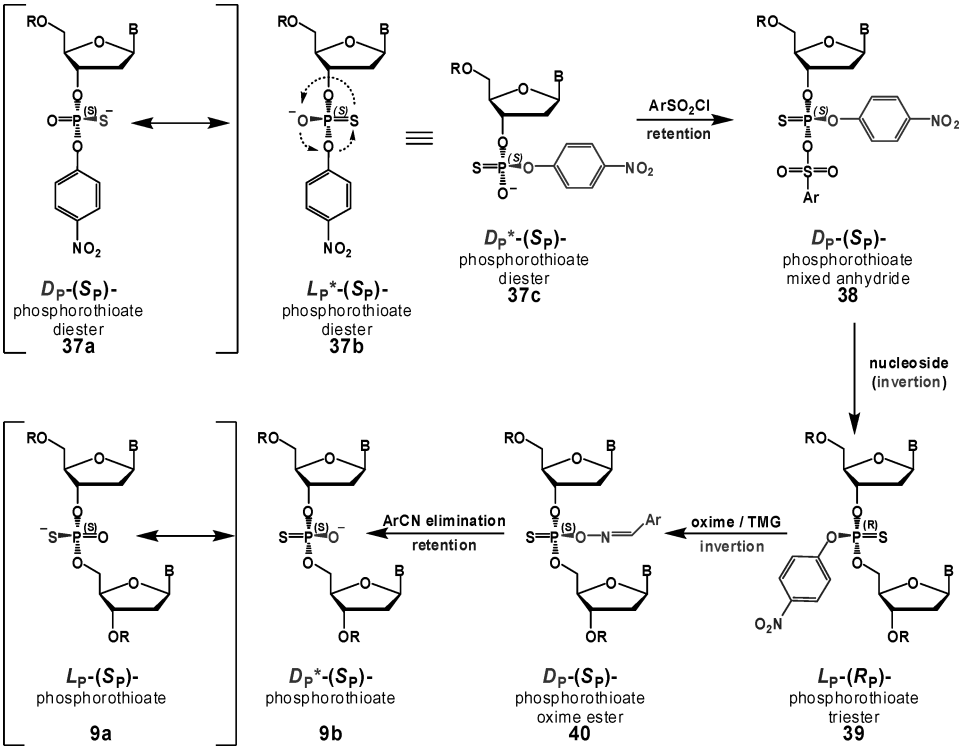

FIGURE 11 Triester approach to dinucleoside phosphorothioates (O-protection).

37b-L_P* with a singly bonded oxygen should be chosen for a convenient tracking the stereochemistry. In order to prevent vertical positioning of the incoming nucleoside, the rotation of the phosphorothioate moiety should be applied at this point. As a result of these two initial manipulations, D_P* (**37c**) projection of D_P (**37a**) phosphorothioate diester **37** is taken as a starting structure. Activation of **37c** with arenesulfonyl chloride leads to the mixed anhydride **38**-D_P (retention), and this may undergo S_N2(P)-type esterification (inversion of configuration**) with formation of triester **39**-L_P. In the next step the aryl moiety in **39** is substituted with an oxime,[10–12] yielding ester **40**-D_P. All these reactions show a correct correlation between stereochemistry and the D_P/L_P descriptors. Finally, elimination of the nitrile moiety proceeds with retention of configuration and yields D_P* diester **9b** (as in the former cases this D_P* form can be formally transformed into a canonical **9a**-L_P phosphorothioate form having proper for the D_P/L_P notation bonding pattern).

**See footnote ‡ on page 6.

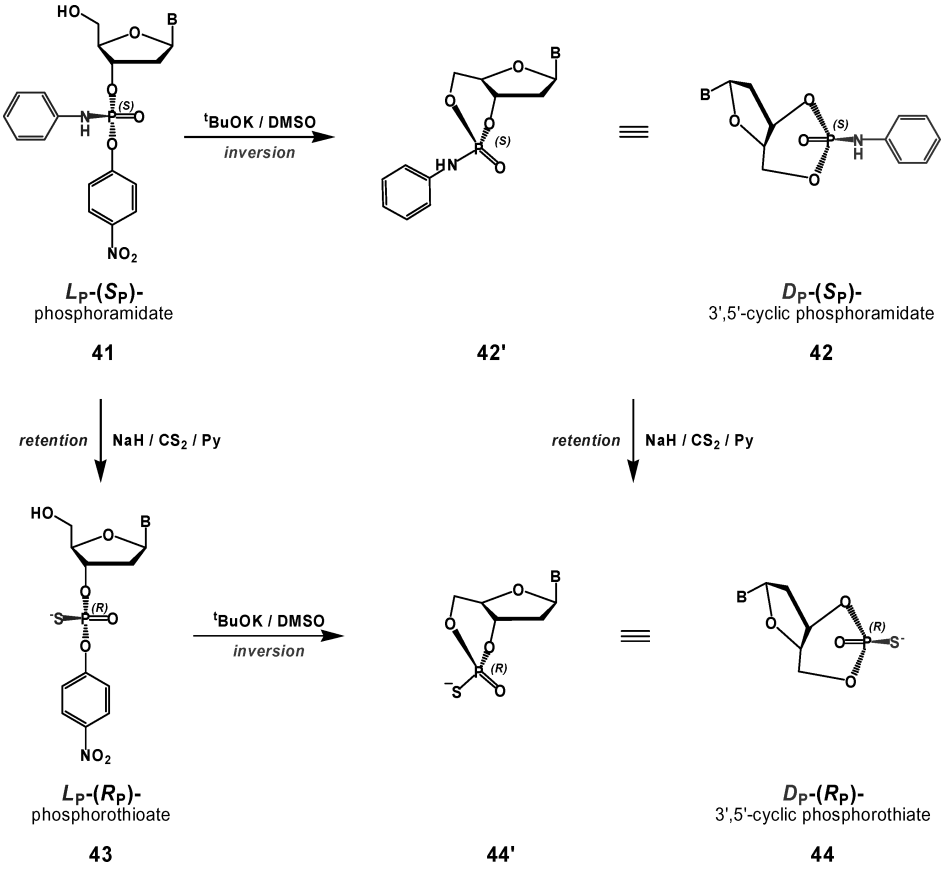

FIGURE 12 Stereochemistry of preparation of cyclic phosphorothioates from aryl phosphoramidates.

A good correlation of R_P/S_P notation with stereochemistry of the reactions also observed in this example is again a coincidence rather than a rule.

Examples of Reactions Involving Cyclic Phosphorus Esters

The next example (Figure 12) shows two approaches to preparing 3′,5′-phosphorothioate **36**-D_P starting from phosphoramidate **41**-L_P.[13] Both can be conveniently followed by the D_P/L_P notation system while the R_P/S_P notation does not inform simply about the stereochemistry of the reaction steps. The presentations of cyclic phosphoramidates as **42′** and **44′** reflecting the intramolecular attack of 5'-hydroxyl at the phosphorus center, with departure of *p*-nitrophenyl moiety, were replaced by projections **42** and **44**, respectively, according to the requirements of the D_P/L_P notation.[3] The D_P/L_P descriptors reflect hence properly the inversion of configuration that occurs during the course of reactions **41** → **42** and **43** → **44**.

CONCLUSIONS

The D_P/L_P notation was found to correlate well with stereochemistry of the analyzed reactions. Usually, retention of configuration at the phosphorus is reflected by no changes of the corresponding D_P/L_P descriptors while the inversion causes a change of the descriptors from L_P to D_P or from D_P to L_P. In some cases, the character of a reaction requires using non-canonical presentations of compounds which are denoted as D_P^*/L_P^*. Such a flexible procedure can be performed on demand in virtually all cases. This allows the stereochemistry of reactions to be properly tracked by the D_P/L_P notation for a vast range of phosphorus esters of nucleosides.

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