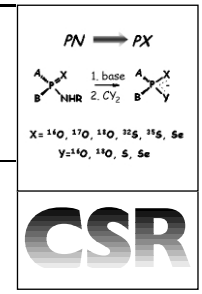
The stereospecific synthesis of P-chiral biophosphates and their analogues by the Stec reaction



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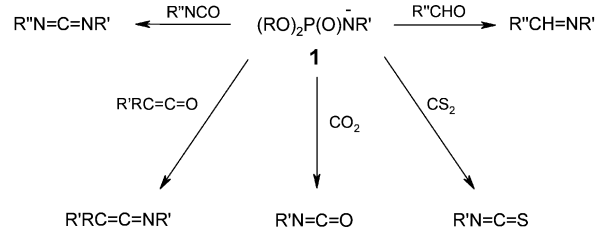
This manuscript summarizes the results of studies on the application of the reaction of dialkyl (aryl) phosphoramidate anions with carbonyl electrophiles for stereospecific synthesis of P-chiral biophosphates (Stec reaction). Following the results obtained with organic phosphoramidates which delineated the scope of the reaction and its stereochemical course, the application of the title reaction is presented for the preparation of diastereomerically pure P-chiral cyclic nucleotide analogues (phosphorothioates, phosphoroselenoates, phosphoroselenothioates, isotopomeric ^{18}O-phosphates), and P-chiral nucleoside monophosphate analogues, as well as dinucleoside phosphate analogues (phosphorothioates, methanephosphonates).

1 Introduction

The Wadsworth–Emmons reaction[1] of dialkyl (aryl) phosphoramidate anions **1** with carbonyl electrophiles and their analogues was developed in the early sixties as a valuable method of preparation of unsaturated nitrogen derivatives, usually of cumulene-type (Scheme 1).



Scheme 1

Interestingly, neither Wadsworth and Emmons nor their followers paid any attention to the other major product of the Wadsworth–Emmons reaction, dialkyl (aryl) phosphates or phosphorothioates, which could otherwise be readily prepared by standard procedures of organophosphorus chemistry. In the

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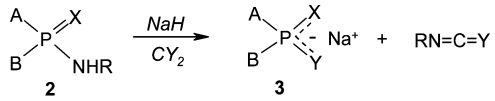
mid seventies, Stec and co-workers were exploring new methodologies leading to the synthesis of P-chiral phosphoramidates. These studies resulted in the successful synthesis of the enantiomerically pure anticancer drugs cyclophosphamide and isophosphamide[2] based upon the separation of diastereomeric cyclic phosphoramidates, where a chiral auxiliary originating from optically active α-phenylethylamine was subsequently removed by hydrogenolysis. Further exploration of synthetic applications of P-chiral phosphoramidates led these authors to re-examine the potential offered by the Wadsworth–Emmons reaction in the synthesis of disubstituted phosphates, phosphorothioates and phosphoroselenoates, with special attention focused upon the stereochemical consequences of this reaction, also called by the authors the PN → PX conversion.[3] It turned out that the development of a synthetic approach involving the preparation of P-chiral phosphoramidates followed by replacement of an amide function by oxygen or sulfur opened the way to the stereospecific preparation of a variety of P-chiral derivatives of phosphorus acids, including numerous biophosphates and their analogues. The potential of such a designed strategy for the stereocontrolled synthesis of these compounds as a new tool for studies of the mode of action of nucleolytic enzymes was noticed early on by Knowles *et al.*,[4] who named the stereospecific PN → PX conversion ***the Stec reaction***. The stereochemistry of the Stec reaction and recent applications of this methodology for the stereospecific synthesis of various P-chiral biophosphates and their analogues will be reviewed in this account.

2 The scope and stereochemistry of the Stec reaction

The original Wadsworth–Emmons procedure[1] was designed for reaction of dialkyl (aryl) phosphoramidates (usually anilidates), first by *N*-metal activation with sodium hydride, followed by carbonyl electrophiles or carbon disulfide to provide the corresponding isocyanates or isothiocyanates (Scheme 1).

Initial studies of Stec and co-workers demonstrated that this procedure could be readily extended to phosphoramidothioates (**2**, X = S) and phosphoramidoselenoates (**2**, X = Se), and that carbon diselenide could also be used as an active electrophile (Scheme 2).[3] Numerous phosphorus acid derivatives, which



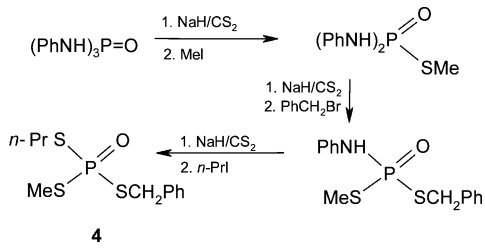
R = alkyl, aryl
A,B = alkyl,aryl, alkoxyl, aryloxyl X = O, S, Se; Y = O, S, Se

Scheme 2

were otherwise relatively difficult to prepare, such as phosphorodithioates (**3**; X = Y = S), phosphoroselenoates (**3**; X = O, Y = Se or X = Se, Y = O), phosphorodiselenoates (**3**; X = Y = Se) or phosphoroselenothioates (**3**; X = Se, Y = S or X = S, Y = Se) became straightforwardly available by this approach.[3]

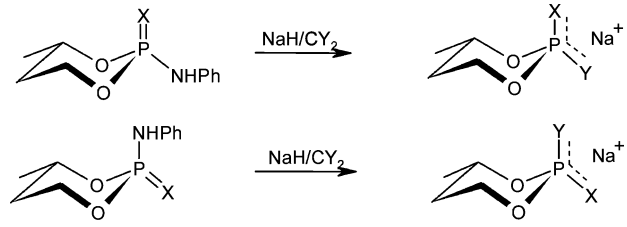
The above procedure was found to be applicable for both acyclic and cyclic (five- or six-membered ring) phosphoramidates, and was further extended to derivatives with one or two carbon–phosphorus bonds, *e.g.* phosphonamidates[3] and phosphinamidates.[5] Phosphorodi- and triamidate derivatives, in addition to phosphoramidates, were also found to follow the same pattern of the PN → PX conversion, as illustrated by the sequential synthesis of asymmetric phosphorotrithioate **4** (Scheme 3).[3]

The stereochemistry of the Stec reaction was first studied by the use of diastereomeric 2-(*N*-phenylamino)-2-*X*-4-methyl-



Scheme 3

1,3,2-dioxaphosphorinanes of known *cis–trans* geometry. It was demonstrated that all the reactions depicted in Scheme 4 proceeded stereospecifically, with retention of configuration at the phosphorus atom.[3]



X = O, Y = S; X = O, Y = Se; X = S, Y = O
X = Se, Y = O; X = Se, Y = S

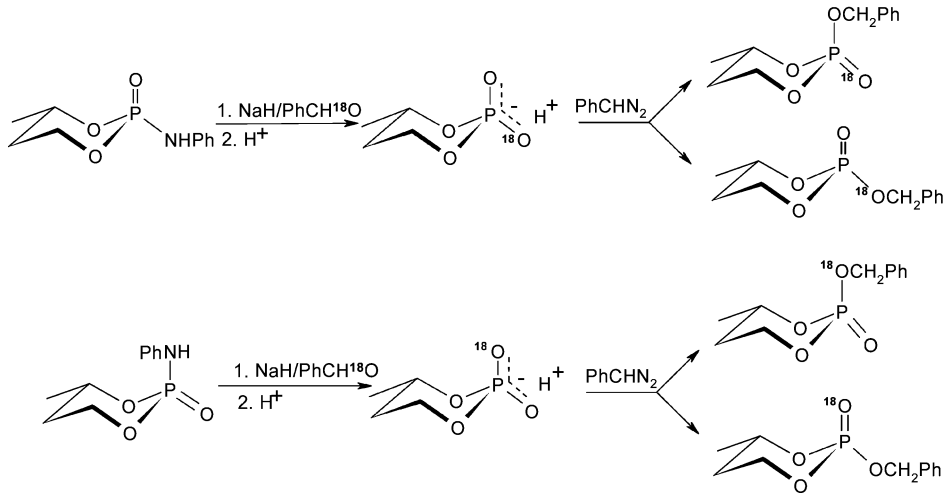
Scheme 4

The cyclic *cis*- and *trans*-2-(*N*-phenylamino)-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes were also employed to determine the stereochemical course of reaction with [^{18}O]-benzaldehyde leading to isotopomeric cyclic phosphodiesters (Scheme 5). The stereochemistry of the PN → P[^{18}O] conversion was examined by mass spectrometry after alkylation of the resulting phosphodiesters with phenyldiazomethane. The reactions were shown to proceed with retention of configuration at phosphorus, with a stereoselectivity higher than 94%.[3]

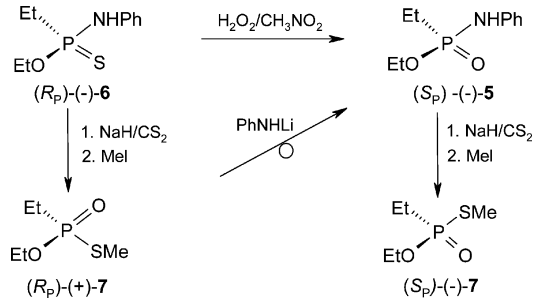
The same conclusion was drawn from the experiments with optically active *O*-ethyl ethylphosphonanilidate **5** and *O*-ethyl ethylphosphonanilidothioate **6**. The stereoretentive course of the Stec reaction, leading to enantiomeric phosphonothioates **7** was assigned by chemical correlation with other reactions of known stereochemistry, *via* the stereochemical cycles[6] depicted in Scheme 6. These reactions include nucleophilic substitution of the thiomethyl group with lithium anilide (inversion),[6] and hydrogen peroxide oxidation of phosphonamidothioate **6** into phosphonamidate **5** (retention).[7]

Further studies carried out with diastereomeric (R_P)- and (S_P)-2-(*N*-phenylamino)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thiones **8** derived from (−)-ephedrine confirmed that the PN → PX conversion also proceeded stereospecifically for five-membered ring phosphoranilidates, with retention of configuration at phosphorus.[3] Again, the absolute configuration of phosphoramidothioate **8** was correlated with that of the corresponding thiolate **9** *via* the stereochemical cycle depicted in Scheme 7.

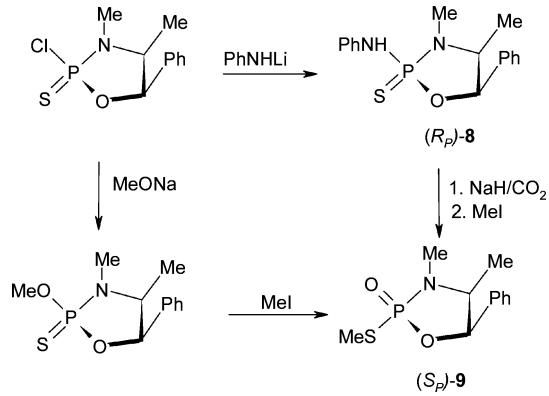
The principle of stereoretention in PN → PX conversion of the five-membered ring phosphoramidates was also extended for the 1,3,2-oxathiaphospholane ring system. (R_P,R_C)-2-[1-(α-naphthyl)ethylamino]-2-thiono-1,3,2-oxathiaphospholane **10** was readily transformed into (R_P)-2-oxo-2-thio-1,3,2-oxathiaphospholane **11** by reaction with potassium hydride and carbon dioxide in DMF (see Scheme 8).[8] The assignment of stereochemistry in the transformation **10** → **11** was established through the determination of the absolute configurations of both **11** (dicyclohexylammonium salt)[8] and **10**[9] by X-ray crystallography. Note that the stereochemistry of the PN → PX conversion involved in this case the reaction of a phosphoramidate other than anilidate.



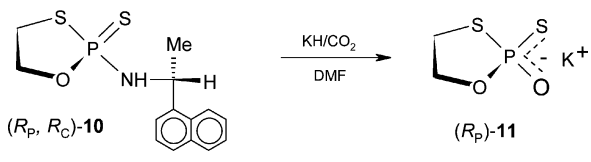
Scheme 5



Scheme 6



Scheme 7



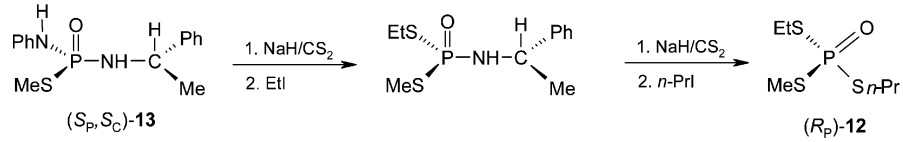
Scheme 8

The stereoretentive PN → PX conversion involving phosphoramidate derivatives of enantiomeric α-phenylethylamine as the chiral auxiliary was used for stereospecific preparation of a series of optically active thio- and/or selenoesters of phosphorus acids.[5] For example, enantiomeric (R_P)-*S*-ethyl-*S*-methyl-*S*-*n*-propyl phosphorotrithioate (**12**) was stereospecifically synthesized starting from the (S_P,S_C)-diastereomer of *S*-methyl-*N*-phenyl-*N*′-(α-phenylethylamino) phosphordiamidothioate (**13**) (Scheme 9). Compound (S_P,S_C)-**13** was isolated by a fractional crystallization of a mixture of diastereomers, and its absolute configuration was determined by X-ray crystallography.[10]

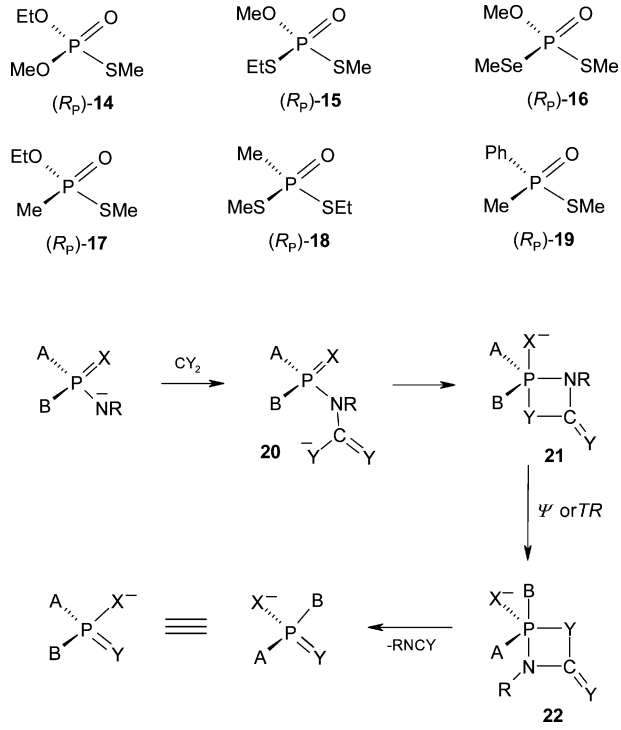
The enantiomers of representative alkyl esters of phosphorothioic (**14**),[8] phosphorodithioic (**15**),[3] phosphoroselenothioic (**16**),[8] methylphosphonothioic (**17**),[8] methylphosphonodithioic (**18**),[8] and methylphenylphosphinothioic (**19**)[8] acids were also prepared in a similar manner. As previously, the appropriate (α-phenylethylamino) phosphoramidate precursors were separated into individual diastereomers by a fractional crystallization and reacted with NaH/CS_2, with successive *S*-alkylation of the resulting phosphorothioate moiety. In similar way as for compound **13**, the absolute configuration of the diastereomeric precursor of phosphoroselenothioate **16** was determined by X-ray crystallography.[11] PN → PS conversion was also successfully applied for the elucidation of the absolute configuration of diastereomeric *O*-menthyl phenyl phosphonanilidothioates.[12]

In the light of these stereochemical results obtained for both acyclic and cyclic (5- or 6-membered ring) compounds, it was postulated that the Stec reaction occurred by the attack of phosphoramidate (phosphonamidate, phosphinamidate, or its thio or seleno analogue) anion at the electrophilic carbonyl (thiocarbonyl, selenocarbonyl) centre (see Scheme 10).[3]

In principle, the resulting adduct **20** may undergo intramolecular rearrangement involving the attack of oxygen (sulfur or selenium) at phosphorus with the formation of pentacoordinated intermediate **21**. Such intermediate undergoes fast polytopal rearrangement (Berry pseudorotation, Ψ, or turnstile rotation, TR) into another pentacoordinate intermediate **22**, involving the shift of the nitrogen substituent into an apical position, necessary for the P–N bond cleavage proceeding according to Westheimer's Rule of apical entry – apical departure.[13,14] The P–N and C–Y bonds are then cleaved



Scheme 9



Scheme 10

synchronously with the formation of the corresponding phosphate (phosphonate, phosphinate, or its thio or seleno analogue) with the retained configuration at phosphorus, and the nitrogen cumulene R–N=C=Y formed as a second product. Such an explanation is in agreement with the rules of nucleophilic substitution at tetracoordinate phosphorus that proceeds *via* pentacoordinate intermediate(s), where the attacking nucleophile approaches phosphorus from the site opposite to the nonleaving group. For such an arrangement of reacting groups, the odd number of polytopal rearrangements (Ψ or TR) within the pentacoordinate intermediate(s) results in retention of configuration at the phosphorus atom.[13,14]

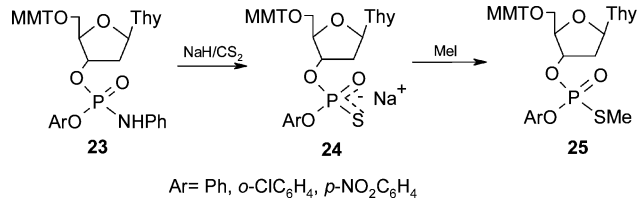
It should be emphasized that although the mechanism presented in Scheme 10 explains well the stereoretentive mode of the PN → PX conversion, no spectroscopic evidence (low temperature ^{31}P NMR studies) was found for the participation of pentacoordinate intermediates **21** or **22** in this reaction.

3 Phosphorothioate analogues of biophosphates

Although the Stec reaction enables the stereospecific synthesis of several classes of P-chiral organophosphorus compounds that are otherwise difficult to prepare, its most important application is to the stereospecific synthesis of P-chiral biophosphates, and in particular P-chiral nucleotide analogues.

The application of P-chiral nucleoside cyclic phosphorothioates to the elucidation of the mode of action of the enzymes involved in nucleic acid metabolism was initiated in the late sixties by Eckstein and Usher who used diastereomeric uridine cyclic (2′,3′)-phosphorothioates to study the mechanism of action of pancreatic ribonuclease.[15] Parallel studies from the Göttingen laboratory were devoted to the preparation of diastereomeric adenosine cyclic (3′,5′)-phosphorothioates.[16,17]

In 1976 Stec was the first to apply the PN → PX conversion to stereospecific synthesis of P-chiral nucleotide analogues.[18] Thus, 5′-*O*-MMT-thymidine-3′-*O*-(*O*-aryl phosphoranilidate) (**23**) was obtained as the result of 3′-*O*-phosphorylation of 5′-*O*-MMT-thymidine with *O*-aryl-*N*-phenyl phosphoramidochloridate. Diastereomers of *o*-chlorophenyl phosphoranilidate **23** were separated by preparative TLC, and each diastereomer **23** was individually treated with sodium hydride followed by carbon disulfide to yield 5′-*O*-MMT-thymidine 3′-*O*-(*O*-*o*-chlorophenyl phosphorothioates) (**24**) (Scheme 11). Subsequently, nucleoside phosphorothioates **24** obtained from diastereomerically pure FAST[19]-**23** and SLOW-**23** were chemoselectively *S*-alkylated with methyl iodide to give the corresponding diastereomerically pure 5′-*O*-MMT-thymidine-3′-*O*-(*O*-*o*-chlorophenyl *S*-methyl phosphorothioates) (**25**). It should be emphasized that the absolute configuration at phosphorus in compounds **23**–**25** was not assigned in these studies.[16]



Ar= Ph, *o*-ClC$_6$H$_4$, *p*-NO$_2$C$_6$H$_4$

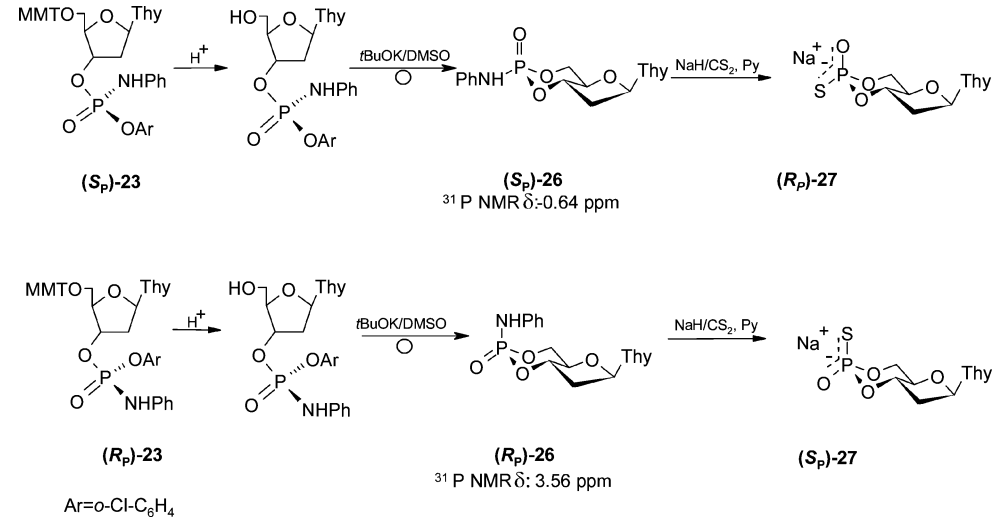
Scheme 11

The anilidates **23** were also found to be convenient precursors for the first preparation of P-chiral nucleoside cyclic (3′,5′)-phosphorothioates by means of the PN → PX conversion. Therefore, the procedures evaluated previously for the stereoretentive conversion of P-chiral phosphoranilidates into the corresponding phosphorothioates[20] were applied to the separated diastereomers of cyclic deoxyribonucleoside phosphoranilidates **26**, which were obtained, after acidic removal of the 5′-monomethoxytrityl protecting group, by the Borden–Smith cyclization[21] of the related acyclic anilidates **23**. In this way, diastereomerically pure thymidine cyclic (3′,5′)-phosphorothioates (**27**) were obtained in satisfactory yields (Scheme 12).

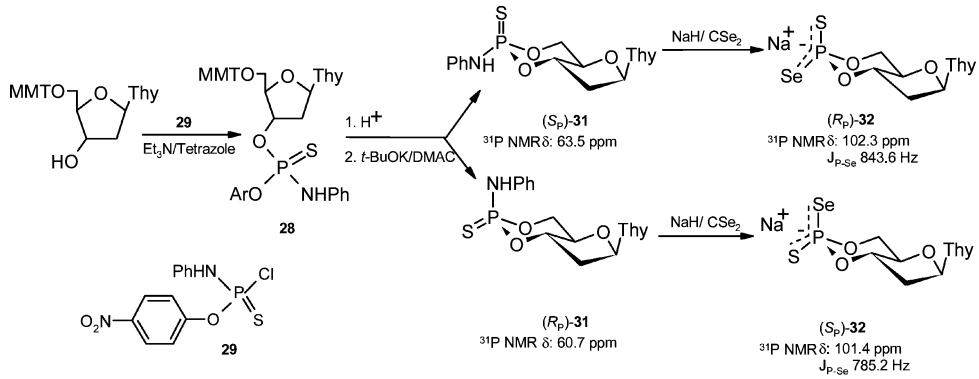
The assignment of the absolute configuration of cyclic phosphorothioates **27** was based on spectroscopic criteria. Since it was known from independent NMR studies that the 1,3,2-dioxaphosphorinanyl part of nucleoside cyclic (3′,5′)-phosphate exists in a chair conformation,[22] the same conformation was assumed for cyclic anilidates **26**. This assumption allows the use of ^{31}P NMR relative chemical shift criteria, which establish that the signals of isomers having an axially oriented exocyclic P–N bond appear at higher field than those with an equatorially oriented phenylamino substituent.[3,23] On this basis, spatial orientation of phenylamino substituents at phosphorus in both diastereomers was tentatively assigned. Consequently, the assignment of the absolute configuration at phosphorus for cyclic phosphorothioates **27** was afforded (Scheme 12). The assignment of P-chirality of acyclic anilidates **23** was based upon the assumption (later confirmed by Gerlt *et al.*)[24] that the ring closure reaction under Borden–Smith[21] conditions occurred with inversion of configuration at the phosphorus atom.

Similar types of acyclic nucleotide precursors, namely nucleoside-3′-*O*-(*O*-aryl *N*-phenyl phosphoramidothioates) (**28**) were used successfully for the stereospecific preparation of a new class of cyclic nucleotide analogues, phosphoroselenothioates **32** (Scheme 13).

Phosphorothioylation of 5′-*O*-MMT-thymidine with *O*-(*p*-nitrophenyl)-*N*-phenyl phosphorothioamidochloridate (**29**) yielded 5′-*O*-MMT-thymidine-3′-*O*-(*O*-*p*-nitrophenyl phosphoranilidothioate) (**28**), easily separable into diastereomers by means of chromatography.[3] The separated (R_P)-**28** and (S_P)-**28** were individually 5′-deprotected, and the corresponding (R_P)- and (S_P) isomers of **30** were treated with an excess of potassium *t*-butoxide, causing cyclization.[21] The resulting isomeric (R_P)- and (S_P)-thymidine cyclic (3′,5′)-phosphoranilidothioates (**31**) were converted in a stereospecific way by treatment with NaH/CSe_2 into (S_P)- and (R_P)-thymidine cyclic (3′,5′)-phosphor-



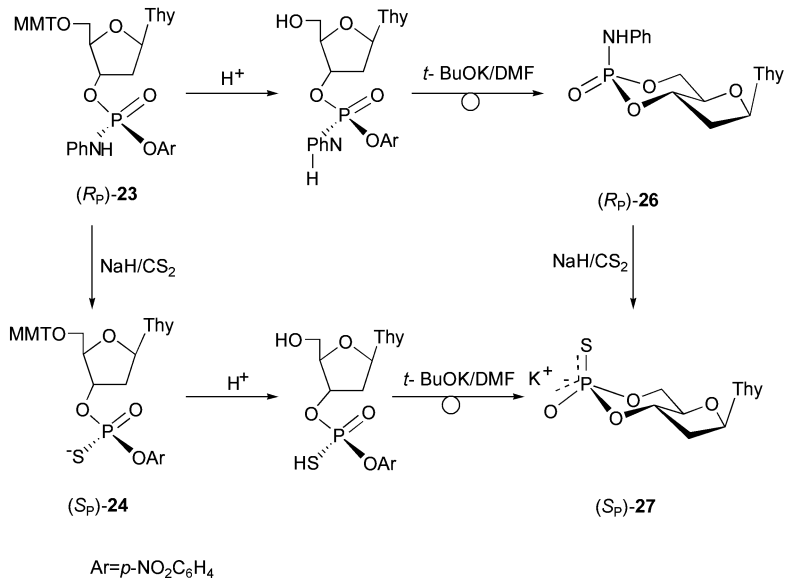
Scheme 12



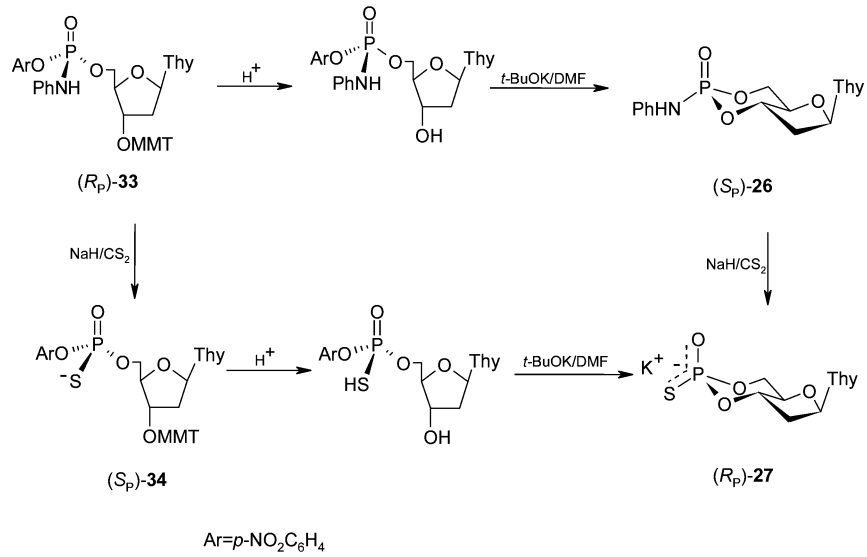
Scheme 13

oselenothioates (**32**), respectively. The absolute configuration of diastereomeric phosphoroselenothioates **32** was assigned on the basis of a previously formulated empirical criterion regarding the stereodependence of the direct spin–spin coupling constants in the family of 2-X-2-Y-1,3,2-dioxaphosphorinanes.[23,25] It was demonstrated, that, for a pair of diastereomers the direct spin–spin coupling constant J_{PX} between phosphorus and an exocyclic, magnetically active nucleus X (^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{77}Se) had a lower absolute value for the isomer with an axially oriented X than for the isomer having X in an equatorial position (Stec′s rule).[26]

In the early eighties, complementary studies were conducted in Stec′s and, independently, in Gerlt′s laboratories, leading to the preparation of diastereomerically pure thymidine cyclic (3′,5′)-phosphorothioates starting from both 3′- and 5′-acyclic phosphoranilidate precursors. Thus, diastereomeric 5′-*O*-MMT thymidine 3′-*O*-(*O*-*p*-nitrophenyl *N*-phenyl phosphoramidates) (**23**)[23,27] and 3′-*O*-MMT thymidine-5′-*O*-(*O*-*p*-nitrophenyl *N*-phenyl phosphoramidates) (**33**)[23,24] were synthesized by phosphorylation of the appropriately 5′-*O*- or 3′-*O*-protected thymidine, respectively, with *O*-*p*-nitrophenyl *N*-phenyl phosphoramidochloridate (Schemes 14 and 15).



Scheme 14



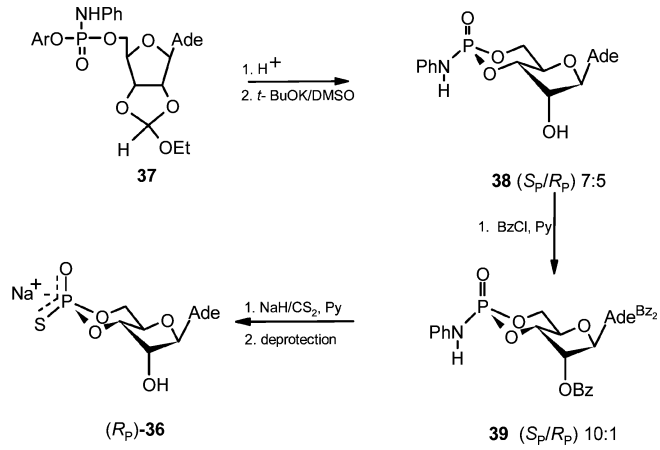
Scheme 15

In both cases the chromatographically separated diastereomeric anilidates **23** or **33** were converted in a stereospecific reaction with NaH/CS_2 into the corresponding acyclic thymidine 3′-*O*-(*O*-*p*-nitrophenyl phosphorothioates) (**24**, Scheme 14), or thymidine 5′-*O*-(*O*-*p*-nitrophenyl phosphorothioates) (**34**, Scheme 15), respectively.[28] Alternatively, the anilidates **23** or **33**, after acidic removal of the monomethoxytrityl group, were cyclized by potassium *t*-butoxide treatment[21] to the corresponding thymidine cyclic (3′,5′)-phosphoranilidates (**26**). Thus, diastereomers (S_P)-**23** and (R_P)-**33** were converted into the equatorial phosphoranilidate **26** of (S_P) configuration, as judged by the low field chemical shift of the product (δ^{31}P NMR = 1.62 ppm), while the (R_P) axial *N*-phenyl phosphoramidate **26**, with a high field chemical shift (δ^{31}P NMR = −1.39 ppm), was obtained from (R_P)-**23** or (S_P)-**33**. The cyclic nucleoside phosphorothioates **27** were prepared either from cyclic anilidates **26** (NaH/CS_2 treatment) or by potassium *t*-butoxide promoted cyclization of phosphorothioates **24** or **34**, after acidolytic removal of the monomethoxytrityl group. The aforementioned NMR-based configurational assignments of cyclic anilidates **26** were further confirmed by enzymatic hydrolysis of the corresponding acyclic phosphorothioates **34** by snake venom phosphodiesterase.[27] This enzyme will hydrolyse only P-chiral nucleoside phosphorothioates of R_P configuration,[29] and was found to specifically digest only phosphorothioate **34** obtained from acyclic anilidate (S_P)-**33**.

The unambiguous assignment of absolute configuration of a series of deoxyribonucleoside cyclic (3′,5′)-phosphoranilidates was accomplished through X-ray crystallographic studies of 2′-deoxyadenosine derivative **35**.[30] It was found that the isomer of **35** that is characterized by high field ^{31}P NMR chemical shift and lower absolute value of ^{31}P–^{15}N coupling constant has the (R_P)-configuration and an axial phenylamino group. Therefore, earlier configurational assignments in the cyclic phosphoranilidate series based on the spectroscopic data[23] and enzymatic digestion[24] were unambiguously confirmed.

The PN → PX conversion was also employed for the stereospecific preparation of (R_P)- and (S_P)-diastereomers of ribonucleoside cyclic (3′,5′)-phosphorothioates. The first chemical synthesis of adenosine cyclic (3′,5′)-phosphorothioate (cAMPS, **36**) was carried out by Eckstein *et al.*[16] In Eckstein preparation, the Borden–Smith cyclization of 5′-*O*-adenosine *O*,*O*-[bis-(*p*-nitrophenyl)] phosphorothioate allowed the authors to isolate only one isomer of cAMPS (later identified as S_P), although it was originally claimed that a mixture of both isomers was obtained.

The first conformationally controlled synthesis leading to diastereomerically pure cAMPS **36** was carried out starting from 2′,3′-ethoxymethylidene adenosine 5′-*O*-(*o*-chlorophenyl-*N*-phenyl phosphoramidate (**37**) (Scheme 16).[3]



Scheme 16

After removal of the 2′,3′-ethoxymethylidene protecting group, the anilidate was subjected to potassium *t*-butoxide assisted cyclization to afford adenosine cyclic (3′,5′)-phosphoranilidate (**38**) in 50% yield, as a mixture of (S_P):(R_P) diastereomers in 7:5 ratio. After treatment of **38** with benzoyl chloride in pyridine the fully protected N^6,N^6,$O^{2'}$-tribenzoyl adenosine cyclic (3′,5′)-phosphoranilidate (**39**) was obtained, albeit in low yield, in the diastereomeric ratio (S_P):(R_P) = 10:1. Treatment of phosphoranilidate **39** with NaH, followed by reaction with CS_2 and the subsequent removal of the protecting groups, provided exclusively (R_P)-cAMPS (**36**).

The reason for this dramatic change of diastereomeric ratio during the treatment of cyclic phosphoranilidate **38** with benzoyl chloride in pyridine was further investigated on model compounds, and was explained in terms of the electrophile-assisted rearrangement of *N*-benzoyl-*O*,*O*-dialkyl phosphoranilidates into *O*,*O*-dialkyl phosphoric-*N*-phenyl iminobenzoates,[31,32] although other explanation can also be offered.

A more successful stereocontrolled preparation of both (R_P)- and (S_P) diastereomers of cAMPS was achieved when commercially available adenosine cyclic (3′,5′)-phosphate (cAMP) was used as a starting material, after protection of the 2′-hydroxy and the exocyclic NH_2 function with benzoyl groups. The resulting N^6,N^6,$O^{2'}$-tribenzoyl adenosine cyclic (3′,5′)-phosphate (**40**) was converted into a diastereomeric mixture (2 : 1) of cyclic (3′,5′)-phosphoranilidates (**39**) in an Appel type reaction[33] with $PhNH_2/PPh_3/CCl_4$. The diastereomers of **39** were separated by preparative thin layer chromatography. Each individual isomer was treated with potassium hydride and CS_2 in DME, and the product of the conversion was finally deprotected with methanolic ammonia yielding diastereomerically pure **36** (Scheme 17).

The use of acetonitrile as the solvent for **40**→**39** conversion afforded the corresponding protected cAMP anilidates **39** in the ratio (S_P):(R_P) = 5:2, in favour of the precursor of (R_P)-cAMPS.[34] By the use of the same methodology, (R_P)- and (S_P)-[^{35}S]-cAMPS were produced when [^{35}S]-CS_2 was taken as an electrophile.[35] The assignment of the absolute configuration of cAMPS (**36**) was based on the application of the chemical shift criterion to diastereomers of cyclic anilidate **39**, and upon the knowledge of the stereochemical course of the Stec reaction.

The assignment of the absolute configuration of (R_P)- and (S_P)-cAMPS **36** made possible comprehensive studies on the mechanism of action of several enzymes responsible for biosynthesis and biodegradation of cAMP, such as adenylate cyclases[36,37] and cyclic phosphodiesterases.[38,39] Since the cyclic phosphates of cytidine, uridine and guanosine have been known to play important biological functions, the methodology involving the synthesis of cyclic anilidates, their separation into diastereomers, and PN → PS conversion was accordingly adapted for the stereocontrolled preparation of their phosphorothioate analogues.[34,40] The spectroscopic assignment of configuration in the series of ribonucleoside cyclic (3′,5′)-phosphorothioates was further confirmed by the determination of the absolute configuration of (R_P)-cUMPS by X-ray crystallography.[41]

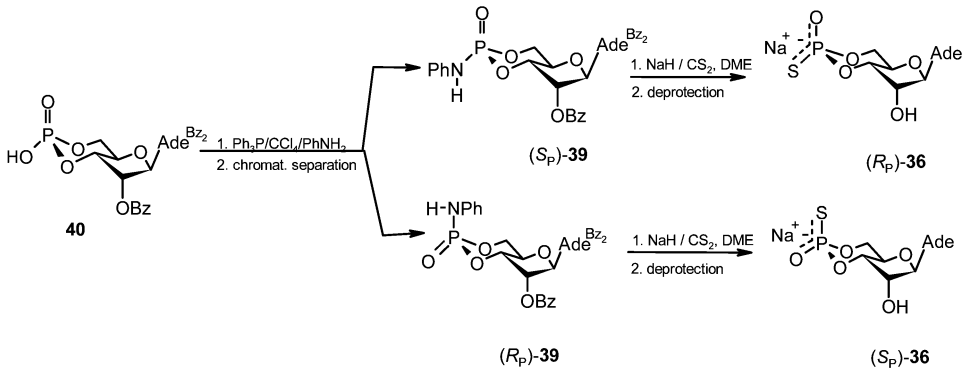
The nucleoside *O*-aryl-*N*-phenyl phosphoramidate precursors **23** were also applied to the synthesis of dinucleoside phosphoranilidates **41**. The deoxyuridine and deoxyadenosine derivatives of type **23** were converted under mild basic conditions into the corresponding 3′-*O*-phosphoranilidates **42** (Scheme 18). These P-prochiral phosphoramidates were reacted with 3′-*O*-acetyl 2′-deoxyuridine in the presence of tris-isopropylbenzenesulfonyl chloride (TPSCl), yielding a diastereomeric mixture of the corresponding dinucleoside (3′,5′)-phosphoranilidates **41**.[3] The activation of **41** with sodium hydride, followed by reaction with carbon disulfide and standard deprotection, afforded a diastereomeric mixture of dinucleoside (3′,5′)-phosphorothioates (**43**). Compound **43** was treated with spleen exonuclease, and both isomers were found to be completely resistant toward this enzyme as observed earlier by Eckstein.[42]

The absolute configuration at the phosphorus centre was assigned enzymatically, employing the stereoselective hydrolytic activity of snake venom phosphodiesterase towards diastereomeric dinucleoside phosphorothioates.[43] Retrospective stereochemical analysis allowed for assignment of the absolute configuration at the phosphorus atom in diastereomers of dithymidine (3′,5′)-phosphoranilidates. Similarly, dithymidine (3′,5′)-phosphorothioate containing 3′-*O*-(*O*-*p*-nitrophenyl phosphoroanilidate) terminal moiety was converted into the corresponding 3′-*O*-(*O*-*p*-nitrophenyl phosphorothioate) by means of the Stec reaction, and used as a substrate for the studies of the stereochemistry of enzymatic transnucleotidylation reaction.[44]

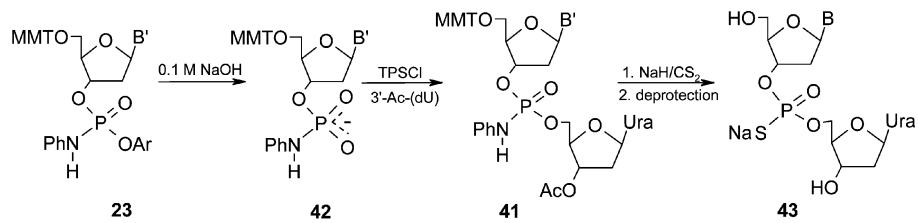
The Stec reaction has also been employed by Gorenstein *et al.*[45] for the conversion of appropriately protected dithymidine (3′,5′)-phosphoranilidothioate (**44**) into the corresponding phosphorodithioate.

4 P-chiral oxygen-isotopomeric biophosphates

Results of the studies of the mode of action of several enzymes catalyzing phosphoryl or nucleotidyl group transfer led to the conclusion that P-chiral phosphorothioate analogues of biophosphates (cNMPS, NTPαS, dinucleoside (3′,5′)-phosphor-



Scheme 17

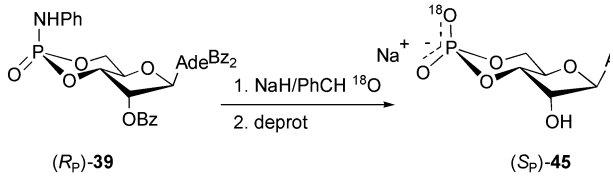


B' = Uracil-1-yl, N^6-benzoyl adenin-9-yl.
B = Uracil-1-yl, adenin-9-yl.

Scheme 18

othioates, *etc.*) could be considered as reliable tools for such studies. However, access to phosphates P-chiral by virtue of oxygen isotopes has been of special interest.[46]

While special stereocontrolled methodologies were elaborated for the synthesis of P-chiral isotopomeric nucleoside monophosphates,[47,48] numerous isotopomeric biophosphates in the phosphodiester series were prepared *via* stereoselective exchange of sulfur by an oxygen isotope starting from previously synthesized P-chiral phosphorothioates.[3,49,50] Some of these compounds were also prepared by the Stec reaction. Thus, P-chiral diastereomeric isotopomers of [^{18}O]-cAMP (**45**) were synthesized for the first time in Stec's laboratory[51] from the protected (R_P)- or (S_P)-adenosine cyclic (3′,5′)-phosphoranilidates **39** by the stereospecific PN → P^{18}O conversion with [^{18}O]-labelled benzaldehyde used as an electrophilic reagent (Scheme 19).



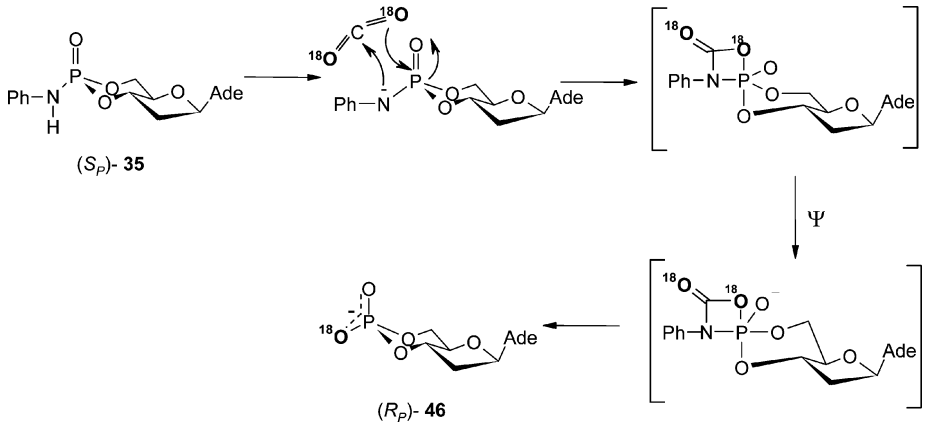
(R_P)-**39** (S_P)-**45**

Scheme 19

In parallel studies, Gerlt and Coderre[52] reported the synthesis of 2′-deoxyadenosine cyclic (3′,5′)-[^{18}O] phosphate (**46**) *via* analogous procedures, starting from the separated diastereomers of deoxyadenosine cyclic (3′,5′)-phosphoranilidate **35**. Each isomer of **35** was activated with NaH, and treated with [^{18}O]-CO_2 to form diastereomerically pure cyclic [^{18}O]-dAMP **46**. From stereochemical data it was unambiguously concluded that the PN → P^{18}O reaction was stereospecific and occurred with retention of configuration. Therefore it was proposed that it proceeded according to the previously formulated pathway (Scheme 20).[3,46]

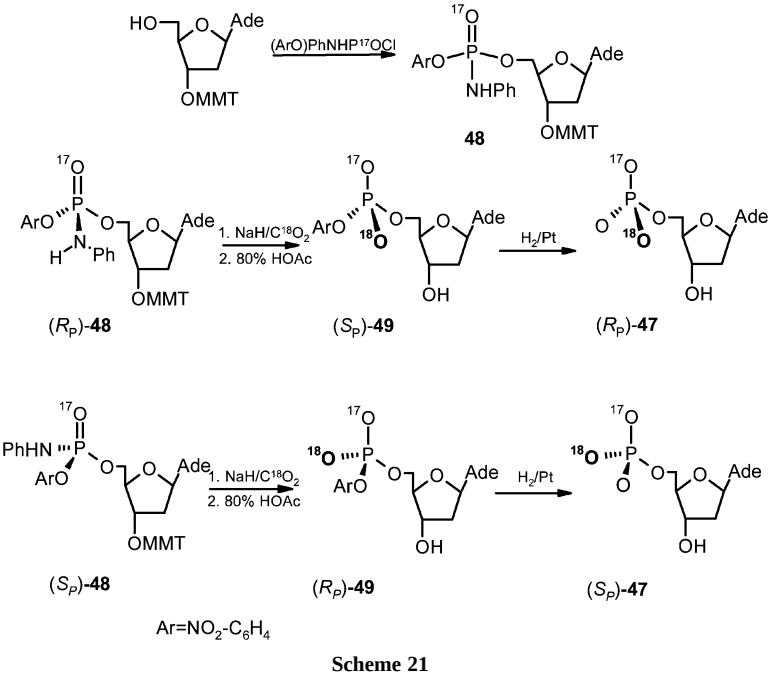
In addition, Gerlt *et al.*[53] successfully extended PN → P^{18}O conversion to the stereoselective preparation of diastereomers of P-chiral isotopomeric thymidine 5′-*O*- [^{16}O,^{17}O,^{18}O]-phosphate (**47**) (Scheme 21).

The authors introduced [^{17}O]-isotope in the reaction of 3′-*O*-MMT-adenosine with [^{17}O]-labelled *N*-phenyl *O*-*p*-nitrophenyl phosphoramidic chloride. The [^{17}O]-labelled anilidate **48** was separated chromatographically into diastereomeric species. Their absolute configurations were tentatively assigned by analogy with the isotopomerically unlabelled anilidates **33**.[24] The [^{17}O]-labelled diastereomerically pure anilidates **48** were separately treated with NaH and [^{18}O]-CO_2, followed by removal of the monomethoxytrityl group. The resulting phosphodiesters (**49**) were hydrogenated in the presence of a platinum catalyst to give adenosine 5′-*O*-[^{16}O,^{17}O,^{18}O]-phosphates **47**.[53] The configurational analysis of isotopomeric phosphates **47** was carried out by ^{31}P NMR following their cyclization into isotopomeric adenosine cyclic phosphates and their subsequent alkylation with methyl iodide.[54] Later on, Gerlt



(S_P)- **35** (R_P)- **46**

Scheme 20

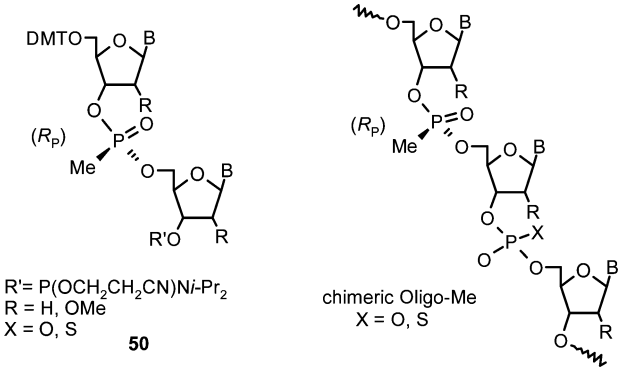


Scheme 21

et al.[46] claimed that in this particular case the PN → P^{18}O conversion was only in part stereoselective and occurred with predominant (60%) retention of configuration at the P-chiral isotopomeric centres. This stereochemical result was in evident discrepancy with numerous results obtained for the PN → PX conversion, where full stereoselectivity was observed. This has never been commented on by the authors in their later publications.

5 Stereocontrolled synthesis of oligo(nucleoside methanephosphonates)

In recent years the Stec reaction has found a novel and interesting application in stereoconvergent synthesis of stereodefined methanephosphonate analogues of nucleic acids that have a predetermined chirality at the internucleotide phosphorus atoms.[55] An internucleotide methanephosphonate linkage is P-chiral. Therefore in the case of oligonucleotides (oligo-PMe) containing *n* methanephosphonate centres obtained *via* elongation of the chain with non-stereospecific coupling reactions, the final product consists of a mixture of 2^n P-diastereomers.



From the experiments carried out with oligomers possessing varying numbers of P–Me bonds of defined chirality it was known that the presence of (R_P)-methanephosphonate linkages improved the binding properties of such oligomers towards complementary DNA or RNA sequences.[56,57] This effect was particularly pronounced for the chimeric dodecathymidylates, assembled from (R_P)-dithymidine (3′,5′)-methanephosphonate monomers (**50**, B = Thy) by the phosphoramidite approach, and possessing in alternate positions phosphodiester internucleotide linkages. It was demonstrated that these chimeric Oligo-Me hybridized with the complementary dodeca(deoxyriboadenylic acid) and dodeca(riboadenylic acid) much more readily than did the corresponding oligomers having incorporated (S_P)-PMe units.[58] From these studies it has become evident that only chimeric oligomers with the incorporated (R_P)-PMe units have acceptable binding affinities towards complementary nucleic acids, and therefore may be of interest as potential antisense therapeutics. Chimeric oligomers built up either from diastereomeric mixtures of dinucleoside (3′,5′)-methanephosphonate units or from those of (S_P)-configuration form significantly less stable duplexes with the same complementary RNA template.[58]

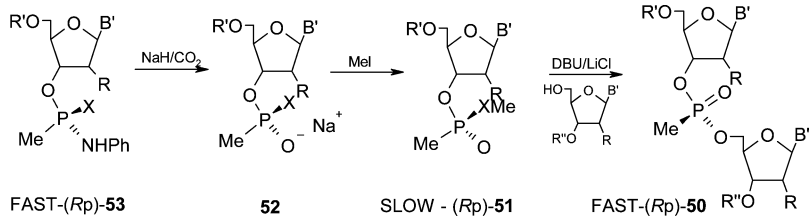
In 1994 a novel stereocontrolled method of preparation of oligo-PMe was reported from Stec's laboratory, based upon the synthesis and separation into diastereomerically pure 5′-*O*-DMT nucleoside 3′-*O*-(*Se*-methyl methanephosphonoselenolates) **51** (X = Se), which in the presence of DBU and LiCl, reacted with 5′-OH nucleosides and/or oligonucleotides in a stereospecific manner.[59] Further evaluation of the above methodology included the use of phosphorothioate monomers **51** (X = S) of both deoxyribonucleosides and 2′-OMe ribonucleosides (Scheme 22).[55,60]

The absolute configuration at the phosphorus atom of each monomer **51** was assigned based on a single-crystal X-ray analysis of the FAST-(S_P) *N*-benzoyl deoxycytidine 3′-*O*-(*Se*-methyl methanephosphonoselenolate) (**51**, R = H, R′ = H, B = Cyt^{Bz}, X = Se) and FAST-(S_P) 5′-O-Pixyl thymidine 3′-*O*-(*S*-methyl methanephosphonothiolate) (**51** R = H, R′ = Px, B = Thy, X = S).[60] It was demonstrated that the condensation that led to the formation of the P–Me internucleotide bond proceeded with inversion of configuration.

Diastereomerically pure *Se* (or *S*)-methyl methanephosphonoselenolates (or -thiolates) **51** have been prepared *via* stereospecific and chemoselective *Se* (or *S*)-methylation of appropriately protected methanephosphonoselenoates (or thioates) (**52**), which, in turn, have been synthesized from the corresponding nucleoside methanephosphonanilidoselenoates (or thioates) (**53**) (the PN → PO conversion) or from nucleoside methanephosphonanilidates (**54**) (the PN → PS conversion). The routes leading to **53** or **54** may involve phosphonylation of the protected nucleosides with $MePCl_2$ in the presence of elemental sulfur or selenium or phosphonylation of 5′-OH and *N*-protected nucleosides with $MeP(S)Cl_2$ or $MeP(O)Cl_2$.[55]

The conversion of the appropriately protected nucleoside methanephosphonanilidoselenoates (or thioates) **53**, or methanephosphonanilidates **54** into the desired methanephosphonoselenoates (or thioates) **52**, considered as precursors for monomers **51**, occurred smoothly in the Stec reaction by means of NaH/CO_2 or NaH/CS_2, respectively. 5′-*O*-DMT thymidine 3′-*O*-(*S*-methyl methanephosphonothiolate) (**51**, X = S, B′ = Thy) was used as a monomer in the condensation with 3′-*O*-acetyl thymidine in the presence of DBU/LiCl.[60] It was established that starting from SLOW-(R_P)-**51**, and using the reaction conditions described in Scheme 23, diastereomerically pure FAST-(R_P)-**50** could be obtained in high yield, while the coupling reaction between FAST-(S_P)-**51** and 3′-*O*-acetyl thymidine provided accordingly SLOW-(S_P)-**50**. Both reactions were stereospecific, and proceeded analogously to those described previously for nucleoside methanephosphonoselenolates **51** (X = Se), that is with inversion of configuration at phosphorus.

All the methods described previously in the literature allow for the preparation of either a mixture of diastereomeric dimers



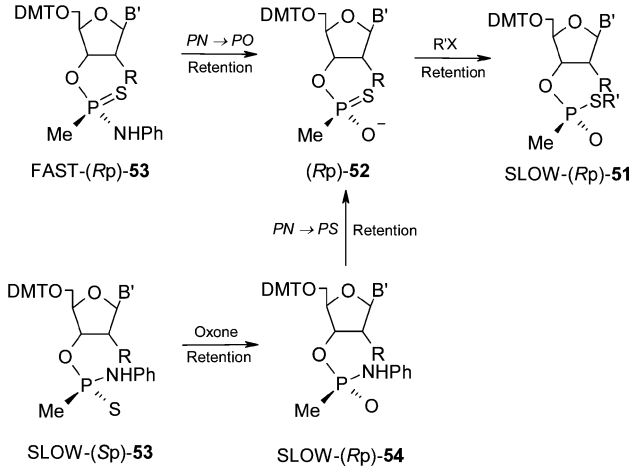
R= H, OMe
B' = Thy, Cyt^{Bz}, Gua^{iBu}, Ade^{Bz}
R' = DMT, Px, H
R" = Ac, TBDMS
X=Se, S

Scheme 22

(to be separated if necessary), or a diastereomeric mixture of monomers. After separation, these can be used in stereospecific coupling reactions leading to particular diastereomeric dimers **50**.[61,62] In each case (S_P)-diastereomers are also synthesized, which are not useful because of their lower affinity towards complementary templates.To avoid the production of 'unwanted waste nucleotides' in the course of antisense studies, Stec *et al.*[55] designed the first ***stereoconvergent*** method that allowed preparation of exclusively (R_P)-**50** from each of the separated diastereomeric precursors of monomers **53** or **54.** This methodology was based upon earlier results that demonstrated the possibility of utilization of (R_P)-5′-*O*-DMT-(*N*-protected) nucleoside 3′-*O*-(*Se*-methyl methanephosphonoselenolates) **51** in the synthesis of (R_P)-**50** and (all-R_P)-oligo-PMe.[59] An alternative route of preparation of *S*-alkyl esters **51** became available also, when methanephosphonanilidates **54** were utilized as precursors. Phosphonanilidates **54** can be transformed by the PN → PS conversion (NaH/CS_2) into sodium salts of nucleoside methanephosphonothioic acids **52**. Moreover, **54** can also be prepared by oxidation of the corresponding methanephosphonanilidoselenoates (or thioates) **53**. The use of diastereomerically pure (R_P)-methanephosphonanilidothioates **53** or (R_P)-methanephosphonanilidates **54** led to the same diastereomer (R_P)-**51**. Therefore both these isomers (R_P)-**53** and (R_P)-**54** became precursors of (R_P)-**50**. However in contrast to anilidothiates (or -selenoates) **53**, the use of anilidates **54** does not give an opportunity to use both separated diastereomers **54** for the exclusive synthesis of (R_P)-**51** (Scheme 23).

The proposed pathway for the stereoselective synthesis of exclusively (R_P)-**51** from both diastereomerically pure precursors, methanephosphonanilidothioates **53**, is depicted in Scheme 24. Isomers (R_P)-**53** are directly used for the preparation of (R_P)-5′-*O*-DMT-(*N*-protected) nucleoside 3′-*O*-(*S*-alkyl methanephosphonothiolates) (**51**), the monomer for (R_P)-**50**. Anilidothioates (S_P)-**53** are stereospecifically oxidized with retention of configuration by means of Oxone® (the mixture containing potassium salt of Caro's acid: $KHSO_4 \cdot K_2SO_4 \cdot 2KSO_5$).[63,64] Using Oxone® under mild and buffered conditions, the so called 'unwanted' isomers (S_P)-**53** can be oxidized with retention of configuration to (R_P) anilidates **54** in high yield.[55] The resulting 5′-*O*-DMT-(*N*-protected) nucleoside 3′-*O*-methanephosphonanilidates (**54**) are converted into 5′-*O*-DMT-(*N*-protected) nucleoside 3′-*O*-methanephosphonothioates (**52**) *via* the stereoretentive Stec reaction (PN → PS).

Further studies confirmed the stereoretentive pathway of the oxidation of P(S) and P(Se) derivatives by Oxone® for both acyclic and cyclic phosphorothio- and phosphoroselenoates.[65] The analysis of Scheme 24 according to the Cahn–Ingold–Prelog rules, and Cram's[6] qualification of stereochemical reactions leads to the conclusion that the sequence of conversions presented here creates an open, 4-membered, diligostatic, antipodal stereochemical cycle with three retentions of configuration at phosphorus and one ligand metathesis, since the phosphorus-bound oxygen atom in (R_P)-**52** originated from transformation either of the phenylamino-function (pathway from R_P-**53**) or of the phosphorothioate sulfur atom (pathway from S_P-**53**).

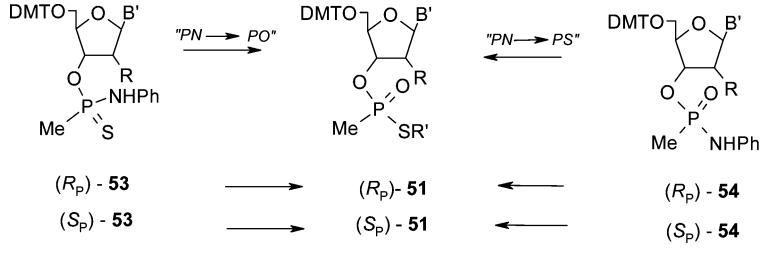


Scheme 24

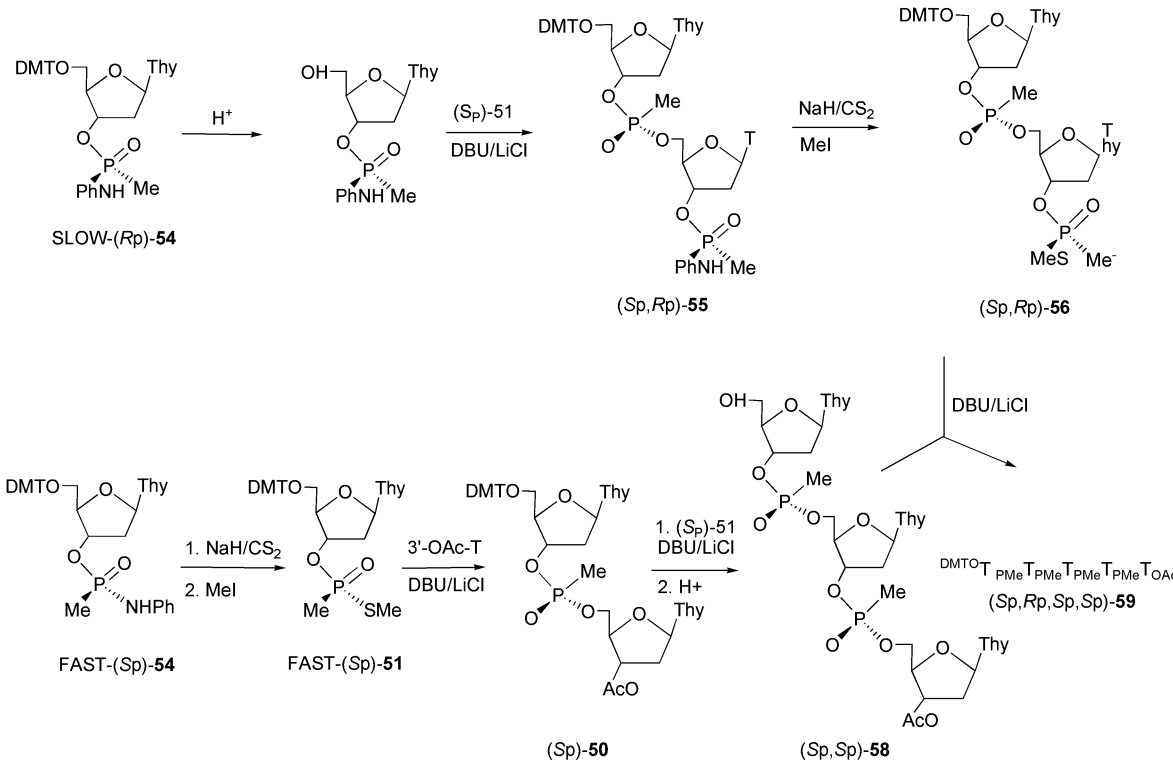
The extension of the scope of this versatile application of anilidates **53** or **54** for 'chemical ligation' of stereodefined oligonucleoside (3′,5′)-methanephosphonates has recently been reported.[66] This approach exploits transient 3′-*O*-methanephosphonanilidate protection with a predetermined chirality at phosphorus, thus enabling the conversion of this methanephosphonanilidate protecting group located at the 3′-end of the stereoregular oligomer **55** into diastereomerically pure 'oligomeric building block' (**56**), which is then available for stereospecific coupling with the 5′-OH group of the other oligonucleotide **57** (Scheme 25). The condensation of diastereomerically pure 5′-*O*-DMT-nucleoside 3′-*O*-(*S*-methyl methanephosphonothiolate) **51** (prepared from diastereomerically pure 5′-*O*-DMT-nucleoside-3′-*O*-methanephosphonanilidate **53** which was used in this synthesis as a common precursor) with 3′-*O*-acetylthymidine provided 5′-*O*-DMT-3′-*O*-acetyl dinucleoside (3′,5′)-methanephosphonate **50**. This condensation was carried out in the presence of DBU/LiCl and occurred with inversion of configuration. Removal of the 5′-*O*-protecting group and further condensation of the resulting 3′-*O*-acetyl dinucleoside (3′,5′)-methanephosphonate with the appropriate *S*-alkyl methanephosphonothiolate **51** enabled the elongation of dinucleotide into 3′-*O*-Ac-protected tri(nucleoside methanephosphonate) (**60**). The DBU/LiCl promoted condensation of **56** with **58** yielded diastereomerically pure penta(nucleoside methanephosphonate) **59** with a predetermined absolute configuration at phosphorus of each internucleotide methanephosphonate linkage, including the one formed in the 'ligation' step.

6 Concluding remarks

During the past twenty five years, the Stec reaction has been broadly recognized as a convenient method of synthesis for



Scheme 23



Scheme 25

several classes of P-chiral organophosphorus compounds, many of them being difficult to prepare by other methods. The preparation of numerous acyclic and cyclic enantiomeric organic phosphates and phosphorothioates and their further derivatization allowed detailed insight into the mechanism of this reaction. The vast majority of these studies revealed full stereospecificity of the conversion, with retention of configuration observed for both cyclic and acyclic organophosphorus derivatives. However, the most significant results have been obtained when the Stec reaction was applied in the field of bioorganic chemistry. This synthetic method gave a straightforward access to a variety of diastereomerically pure P-chiral biophosphates and their analogues, which have been widely used in studies of the mode of action of numerous enzymes including those responsible for transfer of nucleotidyl and phosphatidyl moieties.[46,67] Since a stereochemical criterion has been widely accepted in the studies of the mechanism of action of these enzymes, the availability of diastereomerically pure phosphorothioates (cNMPS, NTPS, dinucleoside phosphorothioates, *etc*.) and the corresponding isotopomeric phosphates cannot be overestimated. Most recent applications of the Stec reaction include a large-scale synthesis of P-chiral non-ionic derivatives of oligonucleotides, which are considered to be candidates for antiviral or anticancer drugs according to the Antisense and Antigene Strategy.[58]

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