carbonyl groups are bound to the titanium particle surface. We do not expect this phenomenon to be a general one and do not expect it to greatly interfere with the utility of the keto ester coupling reaction.

Conclusion and Future Prospects

What began as an accident has now become a reaction of real value in organic synthesis. The titanium-induced coupling reaction of carbonyl compounds allows us to make both symmetrical acyclic olefins from monocarbonyl precursors and unsymmetrical cyclic olefins of all sizes from dicarbonyl precursors. This latter process represents a significant synthetic breakthrough that should continue to lead to the preparation of a large variety of interesting cycloalkenes. Our own continued research on the uses of diketone coupling reactions is leading us in this direction, and we look forward to preparing and studying more unusual compounds such as **19** and **20**.

We have also now learned how to extend the dicarbonyl-coupling reaction to the preparation of large-ring cyclic ketones by titanium-induced cyclization of keto esters. We are continuing to explore this reaction, both is terms of further development as a synthetic method and in terms of use in natural product synthesis. For example, the 14-membered-ring cembrane diterpenes,[29] many of which have considerable biological activity, constitute a large class of synthetically untouched natural products that should be amenable to preparation by our new method. We are actively pursuing these possibilities.

I would like to gratefully acknowledge and sincerely thank my co-workers whose names are listed in the references. Only their patience and skill made this work possible. I would also like to thank the Petroleum Research Fund, administered by the American Chemical Society (Grants 7668-AC1 and 11879-AC1), and the National Science Foundation (Grant CHE 76-06141) for their support of this research.

(29) For a review, see: A. J. Weinheimer, C. W. J. Chang, and J. A. Matson, *Fortschr. Chem. Org. Naturst.*, 285 (1978).

Wadsworth–Emmons Reaction Revisited[1]

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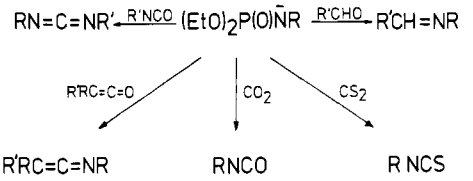
The discovery that organophosphorus ylides and phosphonate anions may interact with carbonyl compounds leading to olefins (Staudinger–Horner–Wittig reaction) had an enormous impact on the development of organophosphorus chemistry. Besides the numerous applications of organophosphorus compounds in organic synthesis, studies on the nature of the Staudinger–Horner–Wittig reaction promoted investigations on the role of pentacovalent phosphorus intermediates and on understanding the behavior of hypervalent phosphorus derivatives.[2] In addition to phosphorus ylides and phosphonates, several phosphoramidates have found broad application as valuable intermediates for the preparation of organic molecules containing the C=N system (Wadsworth–Emmons reaction; Scheme I).[3-5]

Although numerous phosphoramidates have been studied, interest in the synthesis of unsaturated C=N systems has overshadowed interest in the fate of the second product of the Wadsworth–Emmons reaction, namely, dialkyl (aryl) phosphates or phosphorothioates. This can be explained by the fact that these compounds can be easily prepared by other methods such as hydrolysis of tetraalkyl pyrophosphates,[6] oxidation of

Scheme I



dialkyl phosphonates,[7] and base-catalyzed addition of elemental sulfur to dialkyl phosphonates:[8]

$$[(RO)_2P(O)]_2O \xrightarrow{H_2O} 2(RO)_2P(O)OH$$

$$(RO)_2P(O)H \xrightarrow{KMnO_4} (RO)_2P(O)OH$$

$$(RO)_2P(O)H \xrightarrow{S_8,\ base} (RO)_2P(S)O^-BH^+$$

However, the growing interest in the stereospecific synthesis of organophosphates and -phosphorothioates,

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(1) Paper dedicated to Professor Georg Wittig on the occasion of his 85th birthday.

(2) G. Wittig and U. Schöllkopf, *Chem. Ber.*, **87**, 1318 (1954); L. Horner, H. Hoffmann, and A. G. Wippel, *ibid.*, **91**, 61 (1958); G. Wittig in "Organophosphorus Compounds", IUPAC, Butterworths, London 1964, p 245; H. Bestmann in "Phosphorus Chemistry Directed Towards Biology", W. J. Stec, Ed., Pergamon Press, New York, 1980, p 65, and references cited therein.

(3) W. S. Wadsworth, Jr., in "Organic Reactions", Dauben, W. G. Ed., Wiley, New York, 1977, Vol. 25, p 73.

(4) W. S. Wadsworth and W. D. Emmons, *J. Am. Chem. Soc.*, **84**, 1316 (1962).

(5) W. S. Wadsworth and W. D. Emmons, *J. Org. Chem.*, **29**, 2816 (1964).

(6) A. D. F. Toy, *J. Am. Chem. Soc.*, **70**, 3882 (1948); **72**, 2065 (1950).

(7) D. Brown and P. Hammond, *J. Chem. Soc.*, 4229 (1960); A. Zwierzak, *Rocz. Chem.*, **39**, 1411 (1965).

(8) O. Foss, *Acta Chim. Scand.*, **1**, 8 (1947).

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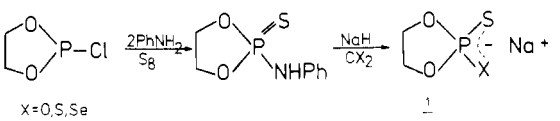
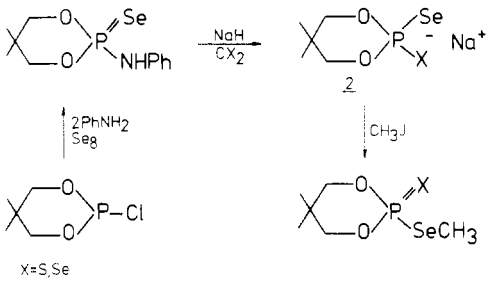
Scheme II



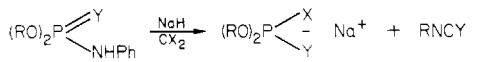
Scheme III



and especially the demonstration of the use of phosphorothioates as tools for investigating the mode of action of numerous phosphoryl transfer catalyzing enzymes,[9] focused the attention of several research groups on methods for the stereospecific preparation of P-chiral phosphates and phosphorothioates. In this laboratory, due to intensive research on biologically active phosphoramidates,[10] studies were undertaken on the application of covalent diastereomeric phosphoramidates for preparation of enantiomeric organophosphates, organophosphorothioates, and organophosphoroselenoates.

Synthesis of Dialkyl Phosphates, Phosphorothioates, Phosphorodithioates, Phosphoroselenoates, Phosphoroselenothioates, and Phosphorodiselenoates

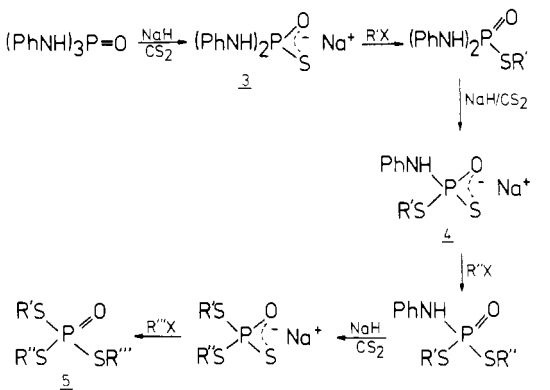
Initially, molecules of simple dialkyl phosphoramidates, derivatives of primary amines, were used. It was found that their treatment with sodium hydride followed by carbon disulfide gives sodium dialkyl phosphorothioates in satisfactory yield.[11] Similarly, the same compounds were obtained when O,O,N-trisubstituted phosphoramidothioates were reacted with base followed by carbon dioxide. This last reaction is of



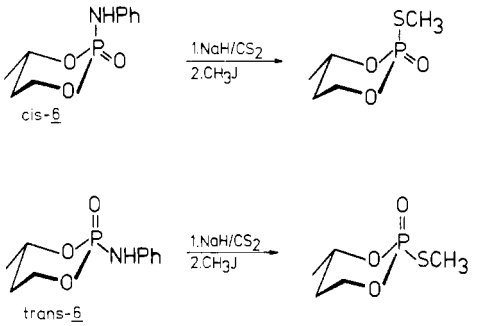
X = O, S, Se; Y = O, S, Se

special value for preparation of small-ring phosphorothioates. For example, 2-chloro-1,3,2-dioxaphospholane reacts smoothly with aniline in the presence of elemental sulfur to give 2-anilino-2-thioxo–1,3,2-dioxaphospholane, which, upon treatment with NaH, may further react with carbon dioxide, carbon disulfide, or carbon diselenide to give the corresponding cyclic phosphorothioates (1, X = O), phosphorodithioates (1, X = S), and phosphoroselenothioates (1, X = Se), respectively[12] (Scheme II). The use of carbon diselenide opened the way to the dialkyl phosphorodiselenoates (2, X = Se),[13] which are difficult to prepare any other

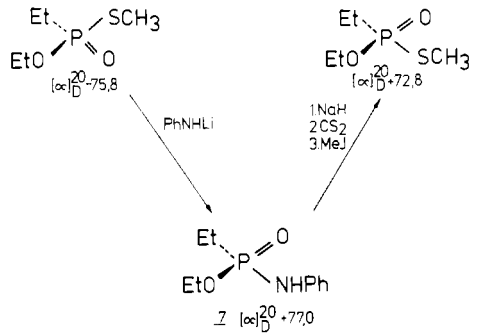
Scheme IV



Scheme V



Scheme VI



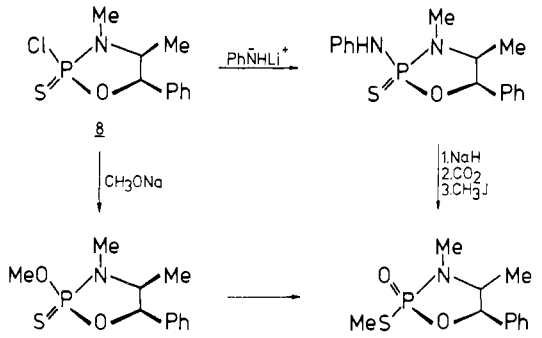
way (Scheme III). The potential of this new approach to the Wadsworth–Emmons reaction is also well illustrated in studies on the synthesis of asymmetric phosphorotrithioates (**5**).[14] Intermediary *N,N'*-diarylphosphordiamidothioates (**3**) and *S*-alkyl *N*-arylphosphoramidodithioates (**4**) can also be isolated in satisfactory yield (Scheme IV).

Stereochemistry of PN → PX Conversion

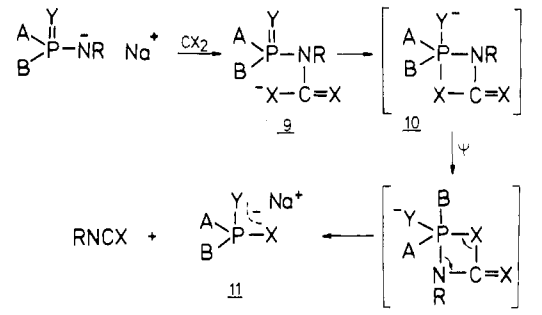
The cheap and readily available diastereomeric 2-(phenylamino)-2-X-4-methyl-1,3,2-dioxaphosphorinanes (**6**)[15] of known cis–trans geometry were used for the preliminary studies on the stereochemistry of PN → PS conversion (Scheme V). These early attempts[11] clearly demonstrated that PN → PS conversion is stereospecific and proceeds with retention of configuration at the phosphorus atom. The same conclusion has been reached from the studies on the optically active *O*-ethyl ethylphosphonamidate (**7**; Scheme VI). Since the elegant work of Inch et al. on the stereochemistry of the

(9) F. Eckstein, *Trends Biochem. Sci. (Pers. Ed.)*, **5**, 157 (1980); P. A. Frey, *Tetrahedron*, **38**, 1541 (1982).
(10) K. Pankiewicz, R. Kinas, W. J. Stec, A. B. Foster, M. Jarman, and J. M. S. Van Maanen, *J. Am. Chem. Soc.*, **101**, 7113 (1979).
(11) W. J. Stec, A. Okruszek, K. Lesiak, B. Uznański, and J. Michalski, *J. Org. Chem.*, **41**, 227 (1976).
(12) K. Lesiak, Ph.D. Thesis, Polish Academy of Sciences, Łódź, Poland, 1980.
(13) K. Lesiak, Z. J. Leśnikowski, W. J. Stec, and B. Zielińska, *Pol. J. Chem.*, **53**, 2041 (1979).
(14) K. Lesiak and W. J. Stec, *Synth. Commun.*, 337 (1977).
(15) W. J. Stec and A. Okruszek, *J. Chem. Soc., Perkin Trans. 1*, 1828 (1975), and references cited therein.

Scheme VII



Scheme VIII

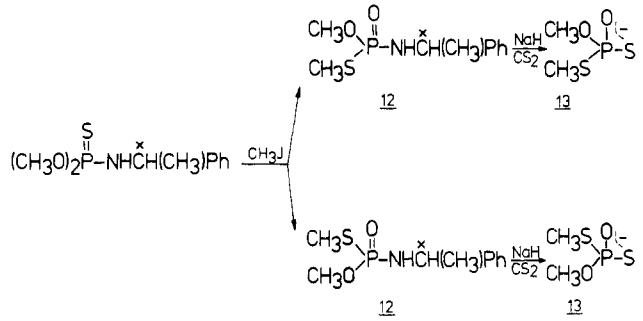


oxazaphospholane ring system,[16] the 2-chloro-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thiones [(2*S*,4*S*,5*R*)- and (2*R*,4*S*,5*R*)-**8**] of known absolute configuration at phosphorus became available. The corresponding 2-anilido-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidine-2-thiones were obtained and further reacted with sodium hydride/carbon dioxide. The resulting sodium phosphorothioates were alkylated without isolation with methyl iodide to give 2-(methylthio)-3,4-dimethyl-5-phenyl-1,3,2-oxazaphospholidin-2-ones (Scheme VII); their absolute configurations were correlated by another method utilizing chemical transformations of known stereochemistry.

As before, the stereochemical correlation of the PN → PX conversion is retention as evident from the reaction sequence shown in Scheme VII.[17] It seems reasonable, in light of results of studies on the stereochemistry of the reaction of the benzylphosphonate anion with benzophenone,[18] that likewise in the process of PN → PX conversion the formation of a pentacovalent intermediate may occur. If, at the first stage, the phosphoramidate anion attacks the carbonyl carbon atom, the phosphorus atom of the resulting adduct **9** undergoes intramolecular attack by means of oxygen (sulfur, selenium) with formation of pentacovalent intermediate **10** (Scheme VIII).

This last process can be regarded as the rate-limiting step. Intermediate **10** undergoes fast polytopal rearrangement that places the P–N bond in an apical position.[19] The synchronous process of PN and CX bond cleavage gives rise to the formation of the corresponding phosphate (phosphorothioate, phosphoroselenoate) and aryl isocyanate (isothiocyanate, isoselenocyanate). It

Scheme IX



should be emphasized that although in the studies on the reaction of **6** with carbon disulfide the ^{31}P low-temperature NMR assay was applied, no evidence for the participation of pentacovalent intermediate **10** was obtained.

According to the established rules of nucleophilic displacements at P(IV) via the P(V) intermediate where the attacking nucleophile approaches phosphorus opposite the nonleaving group, the odd number (1 or 3) of Berry pseudorotations (Berry mechanism) or an odd number (1 or 3) of turnstile rotations (Ramirez mechanism) within the P(V) intermediate explains the observed stereoretentive mode of the Wadsworth–Emmons reaction.[19]

The stereospecificity of the PN → PX conversion allows the application of the Wadsworth–Emmons reaction to the stereospecific synthesis of several chiral phosphates. It has been demonstrated that the most common optically active amine, α-phenylethylamine, can be used in the synthesis of diastereomeric phosphoramidates **12**, which after separation are converted into *O*,*S*-dialkyl phosphorodithioates **13**[20] (Scheme IX). Compounds of that type were formerly prepared via separation of diastereomeric salts of these phosphates with natural bases such as strychnine by means of fractional crystallization,[21] and only recently have Inch and co-workers demonstrated a new approach to the stereospecific synthesis of P-chiral trialkyl phosphorothioates by the process of stereospecific solvolysis of corresponding phosphoramidates.[22]

Preparation of Nucleoside Phosphorothioates

Although methodology based on the Wadsworth–Emmons reaction has not been broadly exploited for the preparation of simple chiral dialkyl phosphorothioates and phosphoroselenoates, its potential can be demonstrated in the preparation of P-chiral biophosphates. Since the pioneering work of Eckstein and Usher on the role of uridine 2′,3′-phosphorothioates in the elucidation of the mode of action of ribonuclease,[23] there has been a growing interest in stereospecific preparation of nucleoside phosphorothioates.[24] The most common approaches applied were the stereoselective enzymatic digestion of diastereomeric mixtures[25] and the stereoselective enzymatic phosphorylation of nucleoside phosphorothioates, e.g., adenosine and guanosine 5′-

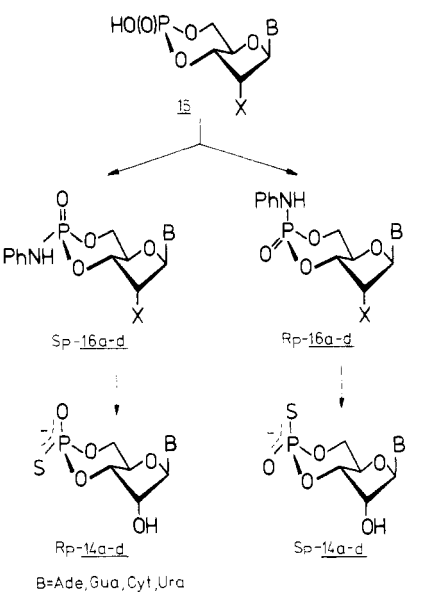
(16) D. B. Cooper, C. R. Hall, J. M. Harrison, and T. D. Inch, *J. Chem. Soc., Perkin Trans. 1*, 1969 (1977).
(17) K. Lesiak and W. J. Stec, *Z. Naturforsch. B*, **33**, 782 (1978).
(18) E. Breuer, S. Zbaida, and E. Segall, *Tetrahedron Lett.*, 2203 (1979).
(19) F. H. Westheimer, *Acc. Chem. Res.*, **1**, 70 (1968); P. Gillespie, F. Ramirez, I. Ugi, and D. Marquarding, *Angew. Chem., Int. Ed. Engl.*, **12**, 91 (1973).
(20) A. Kotyński, K. Lesiak, and W. J. Stec, *Pol. J. Chem.*, **53**, 2403 (1979).
(21) H. Teichmann and Phi trong Lam, *Z. Chem.*, **9**, 310 (1969).
(22) C. R. Hall and T. D. Inch, *Phosphorus Sulfur*, **7**, 171 (1979).
(23) D. A. Usher, D. I. Richardson, and F. Eckstein, *Nature (London)*, **228**, 663 (1970).
(24) F. Eckstein, *Acc. Chem. Res.*, **12**, 204 (1979).
(25) P. M. J. Burgers, F. Eckstein, and D. H. Hunneman, *J. Biol. Chem.*, **254**, 7476 (1979).

phosphorothioates.[26,27] Although the recent developments of modern separation techniques, such as reverse-phase HPLC, offer an alternative route for the preparation of P-chiral nucleoside phosphorothioates,[28] the use of stereospecific methods for their synthesis is still desirable.

Early attempts at the synthesis of adenosine cyclic 3′,5′-phosphorothioates (**14**) based on the Borden–Smith cyclization of adenosine 5′-bis(*p*-nitrophenyl)-phosphorothioates[29] led to a product that had been claimed to consist of a mixture of diastereomers of **14**. However, attempts to separate the mixture into individual R_P and S_P isomers failed. Strikingly, enzymatic digestion of the product claimed to contain both (R_P + S_P) diastereomers of **14** showed no difference in the rates of hydrolysis of both components. In light of the differentiated rate of enzymatic conversion of prochiral 5′-AMPS into ATPαS[26,27] diastereomers and several other examples of stereodifferentiated activity of phosphorylating and nucleolytic enzymes[30] toward P-chiral nucleoside phosphorothioates, it was of interest to reinvestigate the process of hydrolytic behavior of both diastereomers of **14**. A strategy leading to the separated diastereomers of cAMPS has been designed.[31] It is based on the conversion of adenosine cyclic 3′,5′-phosphate **15** (B = Ade) into the mixture of diastereomers of adenosine cyclic 3′,5′-phosphoranilidate **16**, their separation into individual diastereomers, and conversion of each diastereomer in a stereospecific manner into adenosine cyclic 3′,5′-phosphorothioates (**14**, Scheme X).

Among several attempts to prepare the phosphoranilidates **16**, the most successful was the reaction of cAMP with triphenylphosphine/carbon tetrachloride/aniline (Appel reaction).[32] Compounds (R_P + S_P) **16** were obtained in the 45% yield. Their separation has been achieved by means of column chromatography on SiO_2. The Wadsworth–Emmons reaction was applied to these diastereomers and subsequent removal of the protective groups led to the desired (R_P)- and (S_P)-**14**. Assignment of the absolute configuration deserves special comment. Two independent criteria, designed in earlier studies on diastereomeric 2-(phenylamino)-2-X-4-methyl-1,3,2-dioxaphosphorinanes **6**, have been applied, with the preliminary assumption that in both isomers the six-membered dioxaphosphorinanyl part of the molecules **16** exists in a chairlike conformation. First, one relies upon the criterion of the chemical shift in the ^{31}P NMR spectrum: an isomer with an axially oriented phenylamino group absorbs at a higher field than one with an equatorially disposed phenylamino group.[15] Taking into account the fact that the dioxaphosphorinanyl part of the molecule is trans fused to natural D-ribose, elucidation of the spatial orientation of the phenylamino group is equivalent to assignment of the absolute configuration at phosphorus.

Scheme X



Another criterion of absolute configuration assignment was based on the comparison of the absolute value of spin–spin coupling constants between phosphorus and the ^{15}N atom.[31] It has been shown earlier[33] that the axial orientation of the PX bond between phosphorus and another nuclei X of spin number $I = {}^1/_2$ reflects a lower $^1J_{PX}$ absolute value than that for equatorial PX bond. Application of [^{15}N]aniline in the Appel reaction allowed the measurements of PN bond coupling and confirmed the assignment based on chemical-shift criterion. The overall procedure for preparation of both diastereomers of cAMPS is presented in Scheme X.

The synthesis of (R_P)-**14** and (S_P)-**14** opened the way to elucidation of the mode of action of two enzymes responsible for biosynthesis and biodegradation of natural cAMP, adenylate cyclase,[34,35] and cyclic phosphodiesterase,[36,37] respectively. It was found that bovine heart cyclic AMP phosphodiesterase does not hydrolyze adenosine cyclic 3′,5′-(R_P)-phosphorothioate [(R_P)-**14**] (at a rate that can be measured), in contrast with a report that (R_P)- and (S_P)-**14** are about equally good substrates.[29] It is supposed that adenosine cyclic 3′,5′-phosphorothioates obtained from Borden–Smith cyclization of adenosine 5′-bis(*p*-nitrophenyl)-phosphorothioate and used in the original studies were predominantly the S_P diastereomer and not a mixture of S_P and R_P diastereomers as previously thought.

The procedure designed for the stereospecific synthesis of **14** has been used in the preparation of uridine, cytidine, and guanosine cyclic 3′,5′-phosphorothioates[38] and also for the deoxyadenosine cyclic 3′,5′-phosphorothioates.[39] The precursor of the S_P diastereomer of this last compound, deoxyadenosine cyclic 3′,5′-(R_P)-phosphoranilidate, has been studied by means

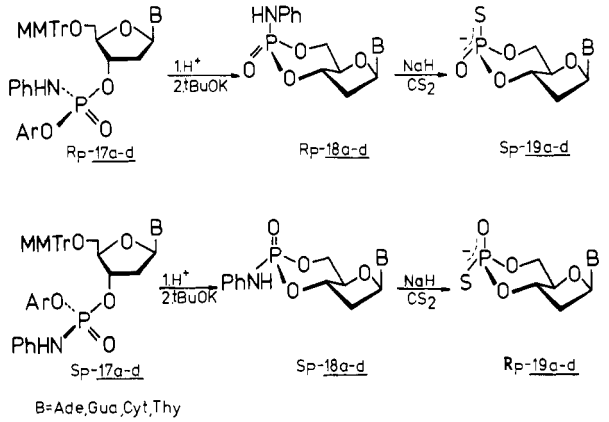
(26) F. Eckstein and R. S. Goody, *Biochemistry*, **15**, 1685 (1976).
(27) K. F. R. Sheu and P. A. Frey, *J. Biol. Chem.*, **252**, 4445 (1977).
(28) R. Bryant, R. D. Sammons, P. A. Frey, and S. J. Benkovic, *J. Biol. Chem.*, **256**, 5965 (1981); D. J. Romaniuk and F. Eckstein, *J. Biol. Chem.*, **257**, 7684 (1982).
(29) F. Eckstein, L. P. Simonson, and H. P. Bär, *Biochemistry*, **13**, 3806 (1974), and references cited therein.
(30) P. A. Frey, J. P. Richard, H. T. Ho, R. S. Brody, R. D. Sammons, and K. F. R. Sheu, *Methods Enzymol.*, **87**, 213 (1982).
(31) J. Baraniak, R. W. Kinas, K. Lesiak, and W. J. Stec, *J. Chem. Soc., Chem. Commun.*, 940 (1979).
(32) R. Appel, *Angew Chem., Int. Ed. Engl.*, **14**, 801 (1975).
(33) W. J. Stec, *Z. Naturforsch. B*, **29**, 109 (1974).
(34) J. A. Gerlt, J. A. Coderre, and M. S. Wolin, *J. Biol. Chem.*, **255**, 331 (1980).
(35) F. Eckstein, P. J. Romaniuk, W. Heideman, and D. R. Storm, *J. Biol. Chem.*, **256**, 9118 (1981).
(36) P. M. J. Burgers, F. Eckstein, D. H. Hunneman, J. Baraniak, R. W. Kinas, K. Lesiak, and W. J. Stec, *J. Biol. Chem.*, **254**, 9959 (1979).
(37) R. L. Jarvest, G. Lowe, J. Baraniak, and W. J. Stec, *Biochem. J.*, **203**, 461 (1982).
(38) J. Baraniak, P. Mejbaum, and W. J. Stec, *Pol. J. Chem.*, in press (1983).
(39) Z. J. Lešnikowski, W. J. Stec, W. S. Zieliński, D. Adamiak, and W. Saenger, *J. Am. Chem. Soc.*, **103**, 2862 (1981).

Table I
Spectral Characteristics of Diastereoisomers of Nucleoside Cyclic 3′,5′-Phosphoranilidates (16, 18) and Nucleoside Cyclic 3′,5′-Phosphorothioates (14, 19)

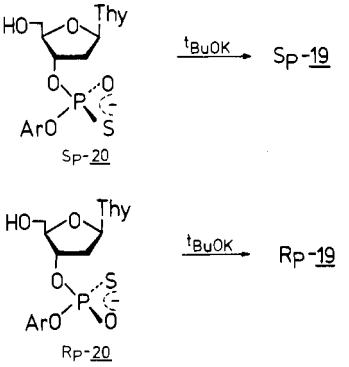
			^{31}P NMR, δ		$^{1}J_{P-^{15}N}$, (Hz)		
no.	B[h]	X	R_P	S_P	R_P	S_P	MS, *m/z*
16a	Ade^{Bz2}	OBz	−3.22[a]	0.81[a]	37.5	49.0	716[g]
14a	Ade	OH	55.19[d]	53.58[d]			561 (M + 3Me$_3$Si)[e]
16b	Gua$^{i\text{-}Bu}$	O-*i*-Bu	−1.44[b]	2.12[b]	39.2	53.7	
14b	Gua	OH	56.15[d]	54.45[d]			
16c	Cyt$^{i\text{-}Bu}$	O-*i*-Bu	−0.64[b]	0.97[b]	38.6	52.7	520[g]
14c	Cyt	OH	55.00[d]	53.34[d]			
16d	Ura	OBz	−1.34[b]	0.54[b]	40.7	52.7	485[f]
14d	Ura	OH	55.09[d]	53.28[d]			
18a	Ade	H	−3.38[a]	0.74[a]	36.7	47.4	388[g]
19a			54.47[d]	52.94[d]			473 (M + 2Me$_3$Si)[c]
18b	Gua	H	−4.43[c]	0.86[c]			404[g]
19b			54.47[d]	52.78[d]			461 (M + 3Me$_3$Si)[e]
18c	Cyt	H	−3.91[a]	0.52[a]			364[g]
19c			54.54[d]	52.70[d]			449 (M + 2Me$_3$Si)[e]
18d	Thy	H	−3.56[a]	0.64[a]			379[g]
19d			54.73[d]	52.11[d]			320 (M − NH$_3$)[f]

[a] Spectra measured in C_5H_5N solution, positive values of chemical shift denoted for compounds absorbing at lower field than 85% H_3PO_4. [b] Spectra recorded in $CHCl_3$ solution. [c] In $HCON(CH_3)_2$. [d] In H_2O. [e] Mass spectra recorded for permethylsilylated compounds. [f] EI-MS of ammonium salt. [g] FD-MS technique. [h] Bz = C_6H_5CO; *i*-Bu = $(CH_3)_2CHCO$.

Scheme XI



Scheme XII



of X-ray crystallography; the results fully confirmed the correctness of the assignment of conformation and absolute configuration at the P atom on the basis of the spectroscopic criteria.[39]

Another approach to the synthesis of nucleoside cyclic 3′,5′-phosphorothioates utilizing the Wadsworth–Emmons reaction is based on the application of phosphorylating reagents bearing the phenylamino function, also designed in this laboratory.[40,41] Phosphorylation of 5′-(monomethoxytrityl)thymidine with *O*-aryl *N*-phenylphosphoramidochloridate led to 5′-(monomethoxytrityl)thymidine 3′-(*O*-aryl *N*-phenylphosphoramidates) (**17**),[42] which after separation into individual diastereomers and removal of monomethoxytrityl group undergo base-catalyzed cyclization to thymidine cyclic 3′,5′-phosphoranilidates (**18**). PN → PS conversion gave (R_P)- and (S_P)-thymidine cyclic 3′,5′-phosphorothioates (**19**)[42] (Scheme XI). This procedure has been utilized in the preparation of deoxyadenosine,[43] deoxycytidine, and deoxyguanosine cyclic 3′,5′-phosphorothioates[44] in this laboratory and also by Gerlt for the preparation of deoxyadenosine cyclic 3′,5′-phosphorothioates, which were further used for elucidation of the stereochemistry of adenylate cyclase catalyzed conversion of dATP into cdAMP.[34]

It is of interest to mention that *t*-BuOK-catalyzed cyclization of thymidine 3′-(*O*-aryl *N*-phenylphosphoramidates) is fully stereospecific and proceeds with inversion of configuration at phosphorus, which rules out in this case the elimination–addition mechanism for nucleophilic substitution at phosphorus incorporating the phosphoramidate moiety.[45] Spectroscopic characteristics of nucleoside cyclic 3′,5′-phosphoranilidates and phosphorothioates are collected in Table I.

The approach based on phosphorylation of nucleosides by means of *O*-aryl *N*-phenylphosphorochloridates allows access to several other types of molecules that are of interest to molecular biology. For example, prior to separation 5′-(monomethoxytrityl)thymidine 3′-[*O*-(*p*-nitrophenyl) *N*-phenylphosphoramidates] can be treated with NaH/CO_2 to give, after 5′-hydroxyl deprotection, thymidine 3′-(*p*-nitrophenyl phosphate), a valuable reagent for enzyme–activity assays.[46] Separated isomers of 5′-(monomethoxytrityl)thymidine 3′-(*O*-aryl *N*-phenylphosphoramidate) are easily con-

(40) W. S. Zieliński and Z. J. Leśnikowski, *Synthesis*, 185 (1976).
(41) W. W. Zieliński, Z. J. Leśnikowski, and W. J. Stec, *J. Chem. Soc., Chem. Commun.*, 772 (1976).
(42) W. S. Zieliński and W. J. Stec, *J. Am. Chem. Soc.*, **99**, 8365 (1977).
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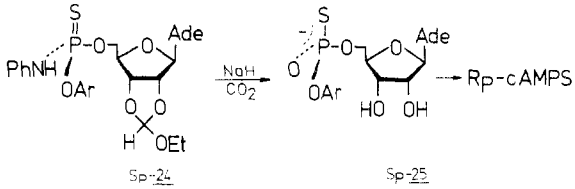
(44) Z. J. Leśnikowski, Ph.D. Thesis, Polish Academy of Sciences, Łódź, Poland, 1981; Z. J. Leśnikowski, W. Niewiarowski, W. S. Zieliński, and W. J. Stec, *Tetrahedron Symposia-in-Print*, in press.
(45) A. F. Gerard and N. K. Hamer, *J. Chem. Soc.* B, 1122 (1967).
(46) W. Niewiarowski, W. J. Stec, and W. S. Zieliński, *J. Chem. Soc., Chem. Commun.*, 524 (1980).

Scheme XIII



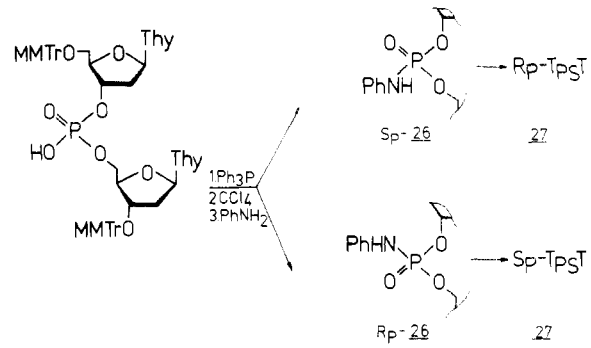
verted to thymidine 3′-(*O*-aryl phosphorothioates) (**20**),[46,47] which were applied successfully by Gerlt[49] in studies on spleen phosphodiesterase. In this laboratory the diastereomers of thymidine 3′-(*p*-nitrophenyl phosphorothioates) (**20**) were cyclized by treatment with *t*-BuOK—these experiments gave the first stereochemical evidence for the stereospecific mode of the Borden–Smith reaction[48] (Scheme XII).

The synthesis and application of *O*-aryl *N*-phenylphosphoramidochloridothioates as a phosphorothioylating reagents opened the way to nucleoside 3′- or 5′-(*O*-aryl *N*-phenylphosphoramidothioates),[13,48] which after separation into individual diastereomeric species, may undergo base-catalyzed cyclization leading to nucleoside cyclic 3′,5′-(*N*-phenylphosphoramidothioates) (**21**). These, by treatment with NaH/carbon dioxide, may be converted to nucleoside cyclic 3′,5′-phosphorothioates (**19**). Application of carbon diselenide to the Wadsworth–Emmons reaction rendered both diastereomers of thymidine cyclic 3′,5′-phosphorothioselenoates (**22**).[13] It is worthwhile to mention that the nucleoside cyclic 3′,5′-(*N*-phenylphosphoramidothioates) (**21**) can be used as the intermediates in the synthesis of nucleoside cyclic 3′,5′-phosphorodithioates, thus far unreported analogues of cNMP. Phosphorothioylation of 2′,3′-(ethoxymethylidene)adenosine with *O*-(*p*-nitrophenyl) *N*-phenylphosphoramidochloridothioate (**23**) led, after deprotection of ribosyl OH groups, to a diastereomeric mixture of adenosine 5′-[*O*-(*p*-nitrophenyl) *N*-phenylphosphoramidothioates] (**24**). Although with difficulty, these diastereomers may also be separated into individual isomers. Reaction of each diastereomer **24** with NaH/carbon dioxide afforded isomers of adenosine 5′-[*O*-(*p*-nitrophenyl) (R_P)- and (S_P)-phosphorothioate] (**25**), respectively[48] (Scheme XIII). Their absolute configuration was assigned recently by Burgers et al.[50] Heretofore only the (S_P)-**25** was available as a pure diastereomer due to the stereoselective hydrolysis of the mixture of (R_P + S_P)-**25** catalyzed by snake venom phosphodiesterase.[50]

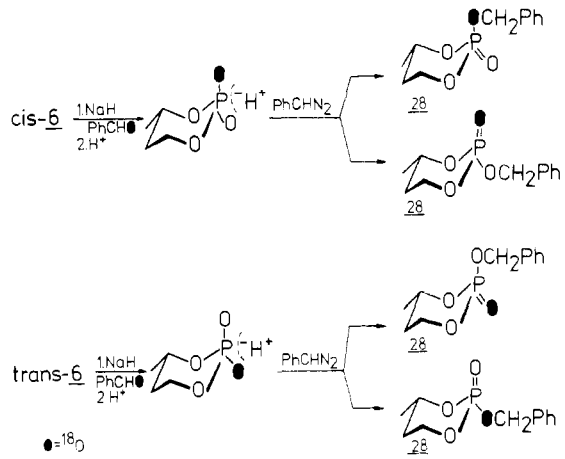
P-Chiral Dinucleoside Phosphorothioates

Although the Wadsworth–Emmons reaction has been applied mostly to the synthesis of mononucleoside phosphorothioates, there are examples of its successful utilization in the preparation of P-chiral dinucleoside

Scheme XIV



Scheme XV



phosphorothioates. Following our earlier work on the synthesis of dithymidyl(3′→5′) *N*-phenylphosphoramidate (**26**),[51] we demonstrated recently its separation into the individual diastereoisomers and conversion by means of the Wadsworth–Emmons reaction into dithymidyl(3′-5′) (R_P)- and (S_P)-phosphorothioates ($T_{PS}T$, **27**)[52] (Scheme XIV). Assignment of the absolute configuration was based on the stereodifferentiated rates of enzymatic digestion. From the earlier independent work of Eckstein[25] and Benkovic[53] it was known that snake venom phosphodiesterase catalyzed hydrolysis of dialkyl phosphorothioates is stereoselective for esters of R_P absolute configuration. Indeed, it has been proven that **27a** [δ_{31P} 55.5, H_2O, obtained from **26a** (δ_{31P} 2.2, $CHCl_3$)] undergoes snake-venom catalyzed hydrolysis much faster than the diastereoisomer **27b** [δ_{31P} 55.1, H_2O; obtained from **26b** (δ_{31P} 1.8)].[52,54] 3′-*O*-(2′-Deoxythymidyl)5′-*O*-(2′-deoxyadenosyl) phosphorothioate [(R_P + S_P)-$dT_{PS}dA$], which was also obtained from the phosphoranilidate precursor, has been used for the elucidation of the stereochemical course of nucleotidyl transfer catalyzed by bacteriophage T7 induced DNA polymerase.[55]

Another important feature of the application of nucleoside phosphoranilidates for the preparation of biophosphates lies in the recent endeavor toward the synthesis of P-chiral nucleosides [^{17}O,^{18}O]phosphates.[56]

(47) Our results indicating that spleen phosphodiesterase (EC 3.1.4.18) catalyzed hydrolysis of thymidine 3′-(4-nitrophenyl (R_P)-phosphorothioates) is stereoselective and leads to thymidine cyclic 3′,5′-(R_P)-phosphorothioate were erronous.[48] Mehdi and Gerlt have shown that the product of this reaction is dithymidyl(3′→5′) phosphorothioate bearing at the 3′ position the 4-nitrophenyl phosphorothioate moiety.[49]

(48) J. Baraniak, Z. J. Leśnikowski, W. Niewiarowski, W. S. Zieliński, and W. J. Stec in "Phosphorus Chemistry: Proceedings of the 1981 International Conference", L. D. Quin and J. G. Verkade, Ed., American Chemical Society, Washington, DC, 1981, ACS Symp. Ser. No. 171, P 77.

(49) S. Mehdi and J. A. Gerlt, *J. Am. Chem. Soc.*, **103**, 7018 (1981).

(50) P. M. J. Burgers, B. U. Sathyanarayana, W. Saenger, and F. Eckstein, *Eur. J. Biochem.*, **100**, 585 (1979).

(51) Z. J. Leśnikowski, J. Smrt, W. J. Stec, and W. S. Zieliński, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, **26**, 661 (1978).

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(53) F. R. Bryant and S. J. Benkovic, *Biochemistry*, **18**, 2825 (1979).

(54) P. A. Bartlett and F. Eckstein, *J. Biol. Chem.*, **257**, 8879 (1982).

(55) R. S. Brody, S. Adler, P. Modrich, W. J. Stec, Z. J. Leśnikowski, and P. A. Frey, *Biochemistry*, **21**, 2570 (1982).

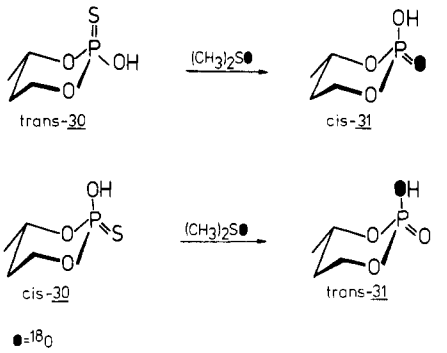
Two elegant methods of the synthesis of monoalkyl [^{16}O,^{17}O,^{18}O]phosphates have been designed in the laboratories of Knowles[57] and Lowe.[58] The phosphoranilidate approach, however, opens the way for the stereospecific synthesis of P-chiral dialkyl phosphates. It has been proved by means of *cis*- and *trans*-2-(phenylamino)-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes (**6**) that their reaction with NaH followed by [^{18}O]-benzaldehyde leads to the desired dialkyl [^{16}O,^{18}O]-phosphates with retention of configuration at phosphorus (Scheme XV).

The stereospecificity of this reaction was shown to be at least 94% by means of mass spectroscopy of the corresponding exocyclic *O*-benzyl esters (**28**). The same reaction with (R_P)-adenosine cyclic 3′,5′-phosphoranilidate gave adenosine cyclic (S_P)-[^{18}O]phosphate.[59] In simultaneous studies Gerlt has proved that the reaction of deoxyadenosine cyclic (R_P)- and (S_P)-phosphoranilidates with NaH/carbon [^{18}O]dioxide leads to adenosine cyclic (S_P)-[^{18}O]- and (R_P)-[^{18}O]phosphates, respectively. The stereospecificity of this reaction has been unambigously proven by means of the ^{31}P NMR isotope shift approach.

It should also be emphasized that in this series of elegant work, Gerlt and co-workers have demonstrated the preparation of the phosphorylating reagent *O*-(*p*-nitrophenyl) *N*-phenyl[^{17}O]phosphorochloridate and demonstated its application to the synthesis of 2′-deoxyadenosine cyclic 3′,5′-[^{17}O]phosphoranilidates, which has opened the way to the stereospecific synthesis of nucleoside cyclic 3′,5′-[^{17}O,^{18}O]phosphates (**29**).[60-62]

Thymidine 3′-(4-nitrophenyl [^{17}O,^{18}O]phosphates) were successfuly used in the elucidation of the mode of action of spleen phosphodiesterase,[30] whereas thymidine 5′-(4-nitrophenyl [^{17}O,^{18}O]phosphates) were used to evaluate the mode of action of snake venom phosphodiesterase.[63] The accessibility of dialkyl phosphorothioates by means of the phosphoranilidate approach recently gained a new dimension: three independent methods of the conversion of nucleoside phosphorothioates into nucleoside [^{18}O]phosphates have been reported. The first one involves transcient formation of phosphorothiocyanatidates that undergo hydrolysis by means of oxygen-labeled water.[64] The second one is based on the reaction of nucleoside phosphorothioates with *N*-bromosuccinimide and immediate hydrolysis of the intermediate by means of oxygen-labeled water.[65] The third method designed by Lowe[66] is based on oxidative brominolysis of phosphorothioates. All reactions

Scheme XVI



seem to be stereospecific and to occur with inversion of configuration at phosphorus. In this laboratory the studies on the stereochemistry of the conversion of dialkyl phosphorothioates (**30**) into dialkyl [^{18}O]phosphates (**31**) by means of dimethyl [^{18}O]sulfoxide are underway (Scheme XVI). Preliminary experiments have shown that under appropriate conditions the stereospecificity of the PS → PO conversion is higher than 90%.[67]

By the latter methods P-chiral oxygen-labeled phosphates are now available, if the corresponding phosphorothioates can be prepared. Their accessibility is greatly enhanced due to the Wadsworth–Emmons reaction utilizing the neutral, easily separable diastereomers of organic phosphoramidates.

Concluding Remarks

The development of stereochemical methods that allows one to gain deeper insight to the mode of interaction between biophosphates and the enzymes responsible for their transformation[56] requires new, efficient, and stereospecific methods for the preparation of model compounds. Although this Account presents only a few examples of the application of the amidodiester approach to the synthesis of P-chiral phosphates and phosphorothioare of biological interest, it is believed that the simplicity of conversion of prochiral diesters of phosphoric acid into chiral phosphoramidates, separation of diastereomers, and easy conversion into desired P-chiral phosphates and phosphorothioates, together with some established methods for determination of the absolute configuration at the P atom, will promote investigations in this new area of bioorganic chemistry. Prochiral phosphates are widely distributed in nature. Nuclei acids, phospholipids, and cell-wall phosphates also undergo numerous biotransformations that await molecular mechanistic elucidation. This new method of "functional protection" of the phosphate moiety and the stereospecificity of the PN → PX conversion seems to provide a general approach to the synthesis of P-chiral phosphates.

The contributions of students and colleagues with whom I had a privilege to work are evident from the references. I owe a debt of gratitude to Prof. S. J. Benkovic, F. Eckstein, P. A. Frey, J. A. Gerlt, D. Gorenstein, J. R. Knowles, and G. Lowe for valuable discussions and availability of manuscripts of their works before publications. I also thank the National Cancer Programme PR-6 and the Polish Academy of Sciences for their generous support.

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