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The Synthesis of Specific Ribonucleotides and Unrelated Phosphorylated Biomolecules by the Phosphoramidite Method

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Contents

INTRODUCTION

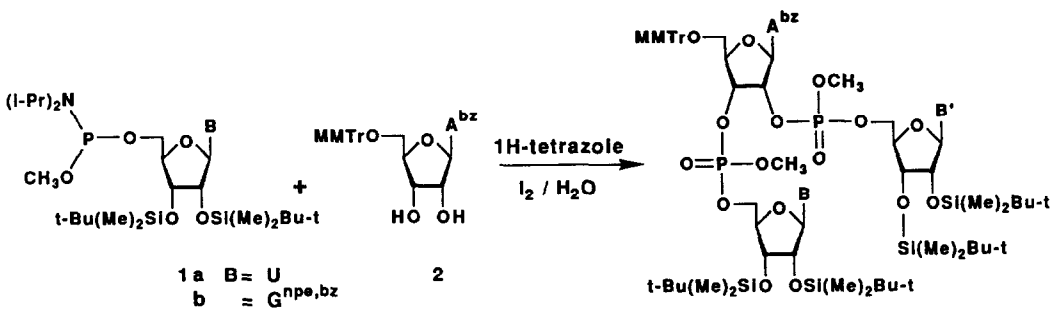
Earlier Reports have dealt with the application of nucleosidic and non-nucleosidic phosphoramidites to the synthesis and functionalization of oligonucleotides and their analogues [1-3] This Report will focus, in part, on the utilization of nucleosidic phosphoramidites in the synthesis of branched RNA structures that play a critical role in the splicing of pre-mRNA and, therefore, in the proper expression of eukaryotic genes. The ability of RNA to catalyze sequence-specific chain cleavage has led to the incorporation of modified ribonucleoside phosphoramidites into RNA in an attempt to define further the structure and function of catalytic RNA molecules Such applications will also be reviewed in this Report.

Furthermore, various phosphorylated biomolecules have been synthesized *via* non-nucleosidic phosphoramidite precursors These include sterol-mononucleotide conjugates, mononucleotide glycoconjugates, phosphosugars, phosphopeptides, glycophosphopeptides, nucleopeptides, phospholipids and their conjugates The synthesis of *myo*-inositol phosphates and their derivatives will be emphasized, as these biomolecules are critically important in the transduction of information in living organisms

1. BRANCHED RNA STRUCTURES AND CATALYTIC RNA MOLECULES

1 1 The Synthesis of Branched RNA Structures

The correct expression of eukaryotic genes depends on the chemical processing (splicing) of pre-mRNA, which involves the accurate excision of introns and ligation of exons The splicing of nuclear polyadenylated RNA occurs with the formation of either a single-stranded circular RNA with a "tail" originating from a branch point (the "lariat" structure in *cis*-splicing reactions)[4] or branches between two linear RNA molecules (the "Y" structures observed in *trans*-splicing reactions) [5] Unlike normal RNA, these structures have vicinal (2'→5')- and (3'→5')-internucleotidic phosphodiester linkages [6] To gain insight into the origin of branch point selection in the splicing process, considerable attention has been directed toward the synthesis of branched RNA oligonucleotides In this context, the preparation of branched structures from phosphoramidite intermediates has been reported by Damha *et al* [7a-b] Their approach consisted of the simultaneous formation of both (3'→5')- and (2'→5')-vicinal internucleotidic linkages Thus, the condensation of the suitably protected ribonucleoside phosphoramidites **1a-b** or the 2',3'-bisphosphoramidite **4** with the appropriate nucleoside **2** or **5** afforded **3a-b** Protection of guanine at *O*-6 was recommended to avoid the formation of side products during the synthesis [7b]

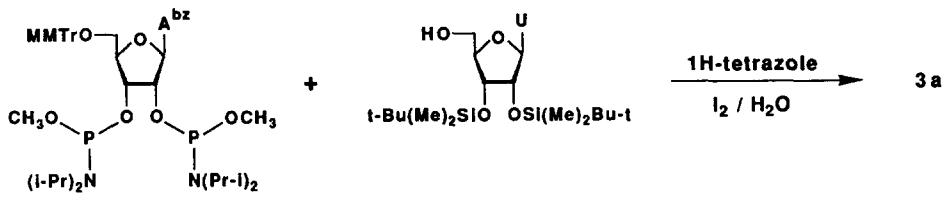


1 a B= U
b = $G^{npe,bz}$

2

3 a B= B'= U
b = B'= $G^{npe,bz}$

MMTr= (4-anisyl)diphenylmethyl
$G^{npe,bz}$= N^2-benzoyl-O^6-(4-nitrophenylethyl)guanin-9-yl
A^{bz}= N^6-benzoyladenin-9-yl, U= uracil-1-yl

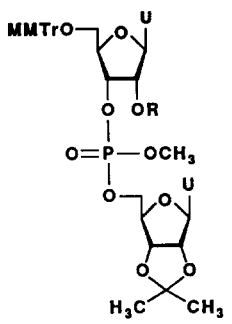


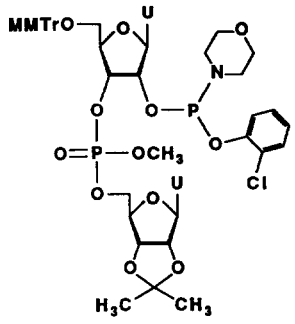
4

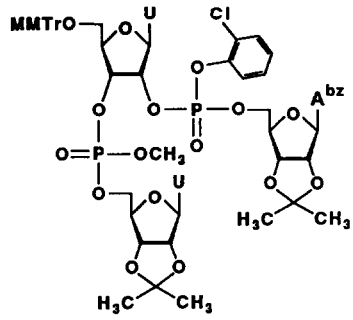
5

3a

In a different approach, Fourrey *et al* [8] demonstrated that the removal of the 2'-*O*-*tert*-butyldimethylsilyl group from the dinucleoside phosphotriester **6a** can be achieved with tetra-*n*-butylammonium fluoride at 0 °C to minimize potential cleavage and/or transesterification of the phosphotriester function The reaction of the intermediate **6b** with 2-chlorophenoxy-bis-(1,2,4-triazolo)phosphine and morpholine led to the phosphoramidite **7** The condensation of N^6-benzoyl-2',3'-*O*-isopropylideneadenosine with **7** and *N*-methylanilinium trichloroacetate generated, after oxidation, the triribonucleoside diphosphate triester **8** [8]





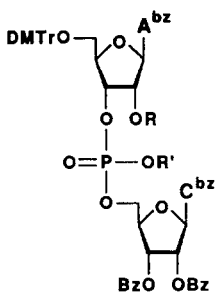


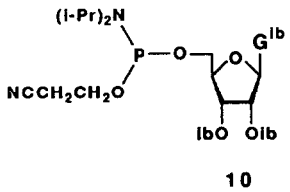
6a R= $Si(Me)_2Bu$-t
b = H

7

8

Caruthers *et al*,[9] have also outlined a simple method for the chemical synthesis of branched RNA structures The dinucleoside phosphotriester **9a** was synthesized by the phosphoramidite approach and was converted to the phosphodiester **9b** upon removal of the cyanoethyl phosphate protecting group with triethylamine The 2'-*O*-*tert*-butyldimethylsilyl group was then cleaved from **9b** with fluoride ion without significant breakage and/or migration of the phosphodiester function The resulting diribonucleoside phosphodiester **9c** was coupled with the appropriate nucleoside 5'-phosphoramidite **10** in the presence of 1*H*-tetrazole, to give the branched oligoribonucleotide **11**[9]





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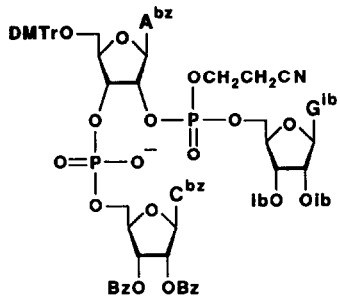


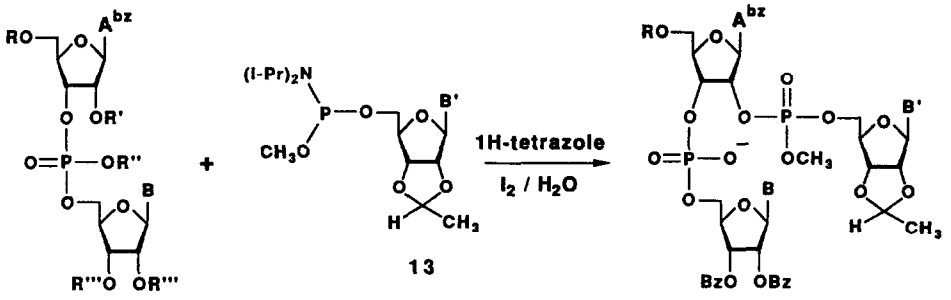
11

9a R= $Si(Me)_2Bu$-t, R'= CH_2CH_2CN
b = $Si(Me)_2Bu$-t, = H
c R= R'= H

DMTr= di-(4-anisyl)phenylmethyl, Bz= benzoyl, Ib= isobutyryl
C^{bz}= N^4-benzoylcytosin-1-yl, G^{Ib}= N^2-isobutyrylguanin-9-yl

Along similar lines, Huss *et al*[10] have reported the synthesis of branched oligomers from the dinucleoside phosphate triester **12a.** Specifically, the 2-chlorophenyl phosphate protecting group of the dimer was first removed with fluoride ion, and then the 2'-*O*-protecting group was cleaved under acidic conditions without affecting the (3'→5')-phosphodiester linkage Condensation of the ribonucleoside phosphoramidite **13** with **12b** afforded, after oxidation, the branched triribonucleotide **14** The deprotected ribonucleotide was resistant to calf spleen phosphodiesterase and ribonuclease T_2 but was completely hydrolysed by snake venom phosphodiesterase [10] A closely related strategy was applied by others to the synthesis of branched tri- and tetraribonucleotides [11,12]

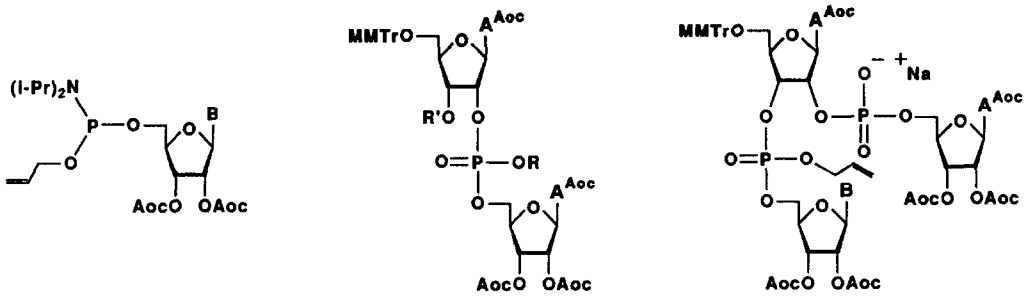
A regiocontrolled synthesis of branched oligoribonucleotides has additionally been described by Hayakawa *et al* [13] Typically, the reaction of the ribonucleoside 5'-phosphoramidite (**15c**) with 5'-*O*-(4-methoxytrityl)-N^6-allyloxycarbonyl-3'-*O*-*tert*-butyldimethylsilyl adenosine yielded the dimer **16** after



12a B= N^4-benzoylcytosin-1-yl, R= 2-(dibromomethyl)benzoyl, R'= 1-methoxytetrahydropyran-1-yl, R''= 2-chlorophenyl; R'''= benzoyl
12b R'= R''= H

14 B= N^4-benzoylcytosin-1-yl, B'= O^6-diphenylcarbamoyl-N^2-propionylguanin-9-yl

oxidation with *tert*-butyl hydroperoxide Selective deallylation of the phosphate function with sodium iodide and desilylation with tetra-*n*-butylammonium fluoride gave the dimer **17** Condensation of **15a-d** with **17** followed by oxidation afforded the branched structures **18a-c** in 76-89% yield based on **17** [13]



15a B= U
b = C^{Aoc}
c = A^{Aoc}
d = G^{Aoc}

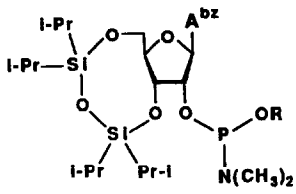
16 R= allyl, R'= $Si(Me)_2Bu$-t
17 = Na, = H

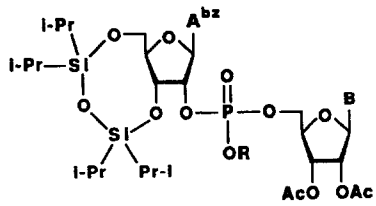
Aoc= allyloxycarbonyl

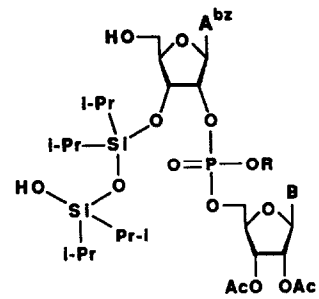
18a B= U
b = C^{Aoc}
c = G^{Aoc}

Zhou *et al* [14a,b] proposed an alternate approach to the synthesis of branched oligoribonucleotides The ribonucleoside phosphoramidites **19a-b** were activated with 1*H*-tetrazole and coupled with a suitably protected ribonucleoside to give the protected dinucleotides **20a-b** which, upon treatment with 0 2 M aqueous hydrochloric acid, afforded **21a-b** Chain extension through the 5'-OH was accomplished by the phosphotriester approach Following phosphate deprotection and desilylation, the ribonucleotide was treated with a ribonucleoside phosphoramidite analogous to **13** to provide the branched tetraribonucleotide **22** [14a,b] This methodology has been slightly modified for the synthesis of a tetrameric branched RNA-DNA structure[15] naturally found in the Gram-negative bacterium *Stigmatella aurantiaca*. The method has also been applied to the preparation of branched penta- and heptaribonucleotides [14c] In a specific case, 2-cyanoethoxy(4-nitrophenylethoxy)-*N,N*-diisopropylaminophosphine has been effective in the phosphitylation of a crucial branch point during the synthesis of a heptameric lariat-RNA [16]

An automated solid-phase synthesis of branched ribonucleotides has recently been developed [17a] A dilute solution (0 01 M) of the ribonucleoside 2',3'-diphosphoramidite **23** was condensed with solid-



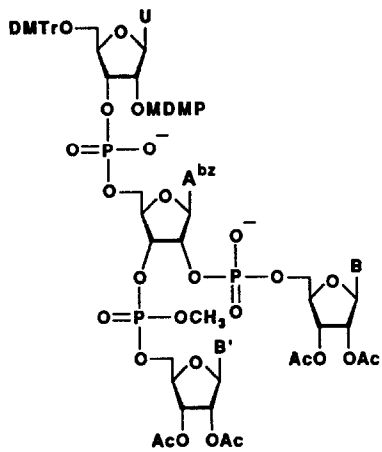




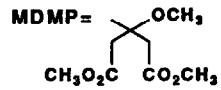
19a R= phenylsulfonylethyl
b = 9-fluorenylmethyl

20a-b

21a-b



22 B= N^2-(4-tert-butylbenzoyl)guanin-9-yl
B'= O^4-(2-nitrophenyl)-2-pyridinone-1-yl

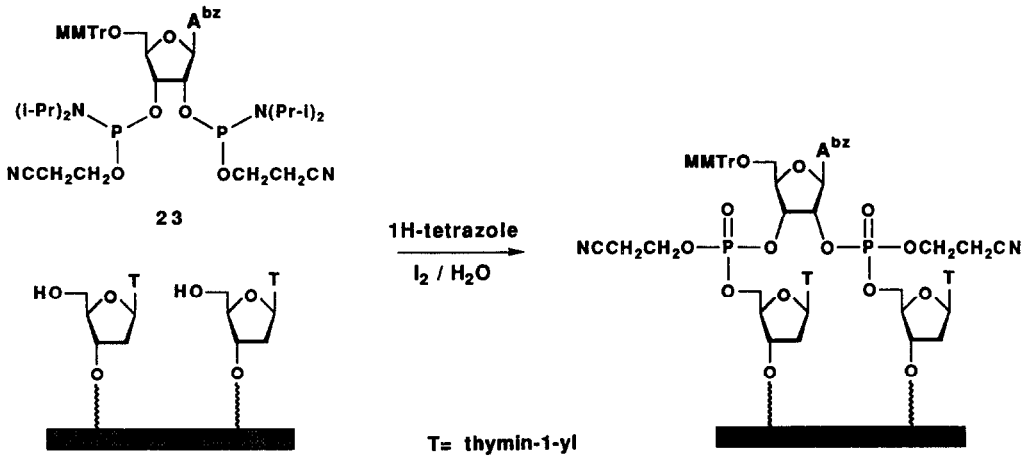


Ac= acetyl

phase bound 2'-deoxythymidine (47 μmole/g LCAA-CPG) (**24**) in the presence of 1*H*-tetrazole to generate the lariat **25** While these conditions led to the almost exclusive formation of **25**, increasing the concentration of **23** (0 075 M) and decreasing the nucleosidic concentration on CPG (7 μmole/g) resulted in the preferential formation of linear dimers rather than the branched trimer [17a] Under optimal conditions, this strategy enabled the synthesis of various branched oligoribonucleotides [17b] Moreover, the preparation of nucleic acid dendrimers, as novel biopolymeric structures, has been accomplished according to this procedure For example, the synthesis of a dendrimer (M W = *ca.* 25,000) having six branched points and twelve terminal ends has been reported [17c]

1 2. Structure and Function of Catalytic RNA Molecules

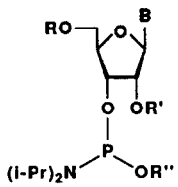
In an attempt to provide a better understanding of the mechanism whereby RNA catalyzes sequence-specific chain cleavage, the incorporation of modified ribonucleoside phosphoramidites into oligoribonucleotides has been necessary In fact, it has been confirmed that the catalytic activity of a RNA enzyme (ribosyme) derived from the Group I *Tetrahymena* self-splicing intron, depended on a wobble base pair rather than a Watson-Crick base pair at the 5'-splice site [18a] This conclusion stemmed from the substitution of a U I wobble base pair for the standard U G wobble base pair which resulted in less effective recognition by the ribosyme It has been argued that while specific features of the bases played some role in splice site recognition, the major component was probably the recognition of the distortion induced in the phosphate backbone by the wobble base pair [18a] The coupling efficiency of the protected inosine phosphoramidite **26** on a 1000Å CPG support was *ca.* 98%.[18a] The



ribonucleoside phosphoramidite **27e** has also been applied to the solid-phase synthesis of oligoribonucleotides to further evaluate the stabilizing effects of wobble base pairs [19]

Of additional interest, modified *Tetrahymena* and *sun* Y self-splicing introns can catalyze the template-directed ligation of RNA oligonucleotides [20a,b] Essentially, tetranucleotides were used as substrates for a primer-extension reaction The 5'-nucleoside of the teramers served as the leaving group while the primer was extended by the remaining three nucleotides When the 5'-nucleoside was guanosine, the primer-extension reaction proceeded efficiently, but competing side-reactions were observed [20c] It was found that the incorporation of the 2'-aminopurine ribonucleoside phosphoramidite **28e**[18b] at the 5'-end of trinucleotides by standard solid-phase methods led to significant reduction of side-reactions, presumably because the modified *Tetrahymena* or *sun* Y ribosyme interacted with 2-aminopurine ribonucleosides with greater affinity than with guanosine [18b,20c]

To instigate structural and functional studies, ribosymes and hammerhead type ribosymes have recently been synthesized by the solid-phase phosphoramidite method The ribonucleoside phosphoramidites **27a-d**,[21] **28a-d**,[22] **29a-d**[23] or **30a-d**[24] were used for these purposes



26 B= O^6-(4-nitrophenylethyl)hypoxanthin-9-yl; R= DMTr, R'= t-Bu(Me)$_2$Si; R''= Me

27a B= U; R= DMTr, R'= tetrahydropyranyl, R''= Me
b = C^{bz}
c = A^{bz}
d = G^{ib}
e = I = hypoxanthin-9-yl

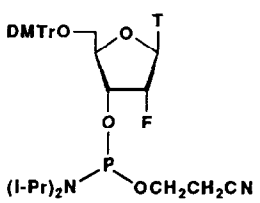
28a-d R= DMTr; R'= t-Bu(Me)$_2$Si; R''= CH_2CH_2CN
28e B= N^2-benzoyl-2-aminopurin-9-yl

29a-d R= levulinyl, R'= tetrahydrofuranyl, R''= CH_2CH_2CN
30a-e = MMTr; = 2-nitrobenzyl; = CH_2CH_2CN

The synthesis of mixed deoxyribo- and ribooligonucleotides with catalytic activity confirmed the involvement of the 2'-OH adjacent to the cleavage site in the substrate and demonstrated that some 2'-OH groups in the catalytic core strongly affected activity [25] Furthermore, mixed DNA/RNA and 2'-*O*-methyl RNA/RNA analogues, derived from the "hammerhead" domain of RNA catalysis, have been synthesized from nucleoside phosphoramidite derivatives to determine the minimum requirement for

catalytic activity It was found that oligodeoxyribonucleotides containing seven to as few as four ribonucleotides were active in cleaving a substrate RNA, albeit at a considerably lower efficiency than that of unmodified ribosymes [26] Interestingly, the 2'-*O*-methylation of ribosyme flanking sequences increased catalytic activity and resistance to nucleases [27] Likewise, chimeric DNA-RNA hammerhead ribosymes demonstrated enhanced catalytic activity *in vitro* and superior stability *in vivo* [28]

The functional role of the native 2'-hydroxyl group of adenosine and guanosine residues in RNA catalysis has been further scrutinized by the incorporation of deoxyribonucleoside phosphoramidites, 2'-fluoro- or 2'-amino-2'-deoxyribonucleoside phosphoramidites (analogous to **31** and **32**, respectively) during solid-phase synthesis of hammerhead ribozymes [29] Ribosymes having every adenosine replaced with 2'-deoxyadenosine or 2'-fluoro-2'-deoxyadenosine showed significantly lower catalytic efficiency compared to unmodified ribosymes However, no single substitution was responsible for the decrease in activity It was concluded that the 2'-OH of the adenosines was not essential for catalysis or for proper formation of the tertiary structure of hammerhead ribosymes [29a] Conversely, the replacement of the 2'-hydroxy function of two guanosines, located in the conserved central core region of the ribosymes, with a 2'-fluoro- or a 2'-amino group reduced the catalytic activity of the corresponding ribosymes by factors of at least 150 or 15, respectively The 2'-amino function can therefore partially fulfill the role of the 2'-OH group in the catalytic core [29c] It is noteworthy that ribosymes containing 2'-fluoropyrimidines at all uridine and cytosine positions were stabilized against nucleolytic degradation in rabbit serum by factors of at least 1000 relative to those of unmodified ribosymes [29b] Furthermore, experiments aimed at cleaving the long terminal repeat RNA of HIV-1 with hammerhead ribosymes indicated that replacing the pyrimidines of a ribosyme with corresponding 2'-fluorocytidines and 2'-fluorouridines together with the incorporation of phosphorothioate linkages at both termini caused only a 7-fold decrease of its catalytic efficiency [29e] The modified ribosyme exhibited, however, a 50-fold increase in stability to hydrolysis by nucleases These results demonstrated the possibility of increasing the resistance of ribosymes to nucleases without severely affecting catalytic activity



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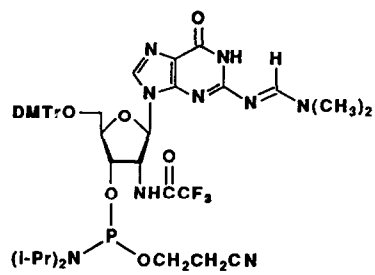
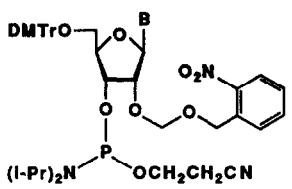


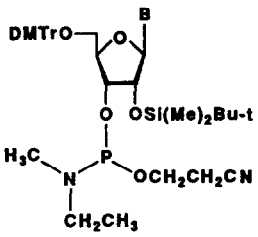
32

The design and application of ribosymes as antisense and therapeutic agents have been reviewed [30] It must be noted that rapid synthesis of oligoribonucleotides using 2'-*O*-(2-nitrobenzyloxymethyl)ribonucleoside phosphoramidites (**33a-d**) has recently been reported by Schwartz *et al.*[31] Oligoribonucleotides (up to 33 bases long) were synthesized using a 0 15 M solution of the phosphoramidites **33a-d** in acetonitrile and a condensation time of 2 min The average coupling yield was better than 98% [31] The efficiency of this method may be attributed to a reduction of steric crowding in the vicinity of the phosphoramidite function with respect to 2'-*O*-(*tert*-butyldimethylsilyl)ribonucleoside phosphoramidite monomers Thus, this methodology should enable the facile and rapid synthesis of ribosymes

Incidentally, the ribonucleoside phosphoramidites **34a-g** have been used in the total chemical synthesis of *E coli* $tRNA^{Ala}$ The phosphoramidites **34a-g** led to coupling yields greater than 98% during a 2 min condensation time on a silica support [32] Triethylamine tris-hydrofluoride was found



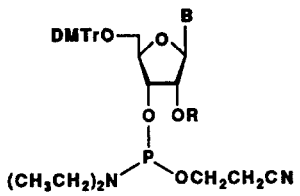
33a B= U
b = C^{bz}
c = A^{bz}
d = G^{ib}



34a B = uracil-1-yl
b = uracil-5-yl
c = thymin-1-yl
d = 5,6-dihydrouracil-1-yl
e = N^4-acetylcytosin-1-yl
f = N^6-phenoxyacetyladenosin-9-yl
g = N^2-phenoxyacetylguanin-9-yl

more effective than tetra-*n*-butylammonium fluoride for the complete removal of 2'-*O*-silyl protecting groups [32]

The ribonucleoside phosphoramidites **35a-d** have similarly been utilized in the synthesis of oligoribonucleotides (up to 74 bases long) [33] The condensation time varied between 5 min to 16 min depending on the phosphoramidite used; and the coupling efficiency averaged 97-99%.[33]



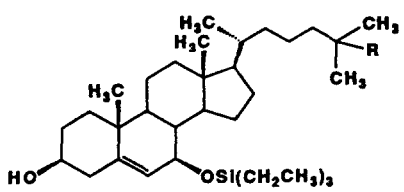
35a B= U, R= Si(Me)$_2$Bu-t
b = C^{bz}, = Si(Me)$_2$Bu-t
c = A^{bz}; = Si(Me)$_2$Bu-t
d = G^{bz}, = Si(Pr-i)$_3$

Phosphoramidite derivatives have also been applied to the phosphorylation and functionalization of unrelated biomolecules Such applications will be discussed in the following section

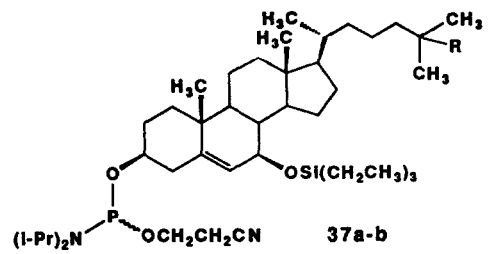
2. PHOSPHORYLATED BIOMOLECULES

2 1 Sterol-mononucleotide Conjugates.

Several cholesterol derivatives including 7ß-hydroxycholesterol, 7ß,25-dihydroxycholesterol, and 7α,22(*S*)-dihydroxycholesterol are cytotoxic to tumor cells *in vitro* [34a] The high lipophilicity of these oxysterols complicated *in vivo* studies Consequently, the coupling of oxysterols derivatives with nucleoside analogues through a phosphodiester linkage could simultaneously enhance the hydrophilicity of oxysterols and the lipophilicity of nucleosides Furthermore, the hydrolysis of these amphiphilic molecules under physiological conditions may lead to the formation of nucleoside 5'-phosphates, which are the active form of antitumoral nucleosides [34b] Ji *et al.*[35] reported the preparation of the phosphoramidites **37a-b** from the reaction of the sterols **36a-b** with (2-cyanoethoxy)-bis-*N,N*-diisopropylaminophosphine and *N,N*-diisopropylammonium tetrazolide

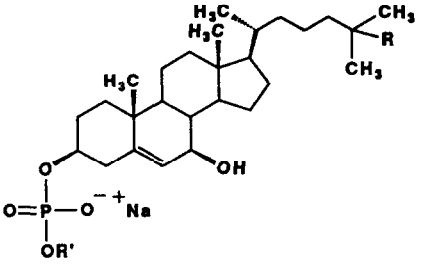


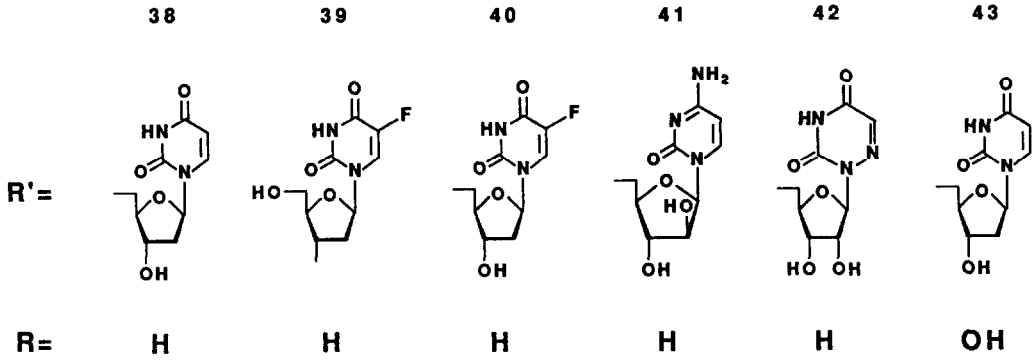
36a R= H
b = OH



37a-b

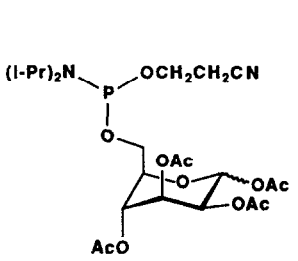
The reaction of the phosphoramidites **37a-b** with suitably protected nucleoside analogues and 1*H*-tetrazole followed by oxidation with *m*-chloroperoxybenzoic (MCPBA) afforded the corresponding phosphotriester conjugates The purified sterol-nucleoside phosphate diester analogues **38-43** were isolated in yields greater than 60%[35]



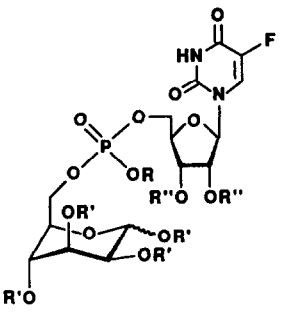


2.2 Mononucleotide Glycoconjugates

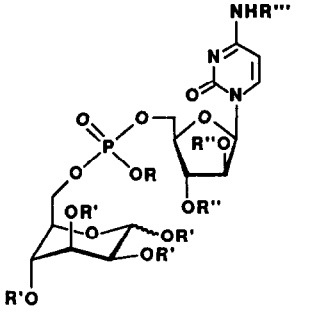
The synthesis of lipophilic phosphate triester derivatives of 5-fluorouridine and arabinocytidine, as anticancer prodrugs, has been achieved by Le Bec and Huynh-Dinh[36] Specifically, the phosphoramidite **44** was synthesized and coupled with the 5'-hydroxy function of properly protected 5-



44



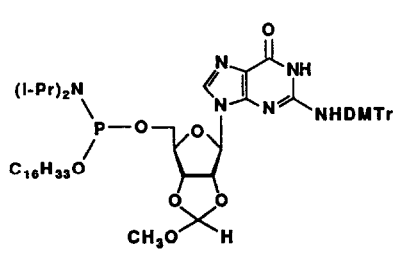
45 R= CH_2CH_2CN;
R'= Ac; R''= Bz
46 R= n-Bu_4N^+; R'= R''= H
47 = $C_{16}H_{33}$; = R''= H



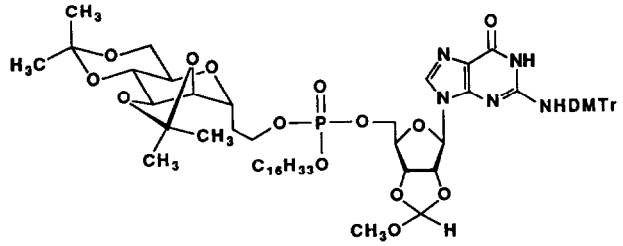
48 R= CH_2CH_2CN,
R'= Ac; R''= R'''= Bz
49 R= n-Bu_4N^+;
R'= R''= R'''= H
50 R= $C_{16}H_{33}$;
R'= R''= R'''= H

fluorouridine and arabinocytidine derivatives The nucleoside phosphotriesters **45** and **48** were subsequently isolated, saponified, and converted to the phosphodiesters **46** and **49** in *ca.* 90% yield The reaction of **46** and **49** with halohexadecanes afforded the lipophilic phosphotriesters **47** and **50** [36] These conjugates may passively permeate unilamellar vesicles and enhance their potency against cancer cells both *in vitro* and *in vivo*

The synthesis of a guanosine 5'-diphosphate mannose (GDP-Man) analogue as a potential inhibitor of glycosyltransferases has been described by Broxterman *et al* [37] Glycosyltransferases are essential enzymes in the biosynthesis of glycoconjugates involved in biological processes such as cell-cell recognition, cell growth and differentiation [38] The protected ribonucleoside phosphoramidite **51**, prepared from N^2-(4,4'-dimethoxytrityl)-2',3'-*O*-(methoxymethylene)guanosine and bis-(*N,N*-diisopropylamino)hexadecyloxyphosphine, was activated with 1*H*-tetrazole and combined with 3,7-anhydro-2-deoxy-4,5 6,8-di-*O*-isopropylidene-D-*glycero*-D-*talo*-octitol Oxidation of the resulting phosphite triester with *tert*-butyl hydroperoxide gave the phosphotriester **52** in 65% yield [37] No data pertaining to the inhibitory effect of the deprotected GDP-Man analogue on the biosynthesis of glycoconjugates was reported

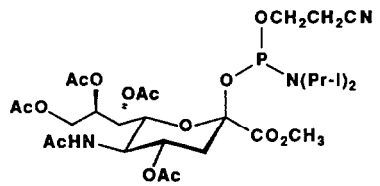


51

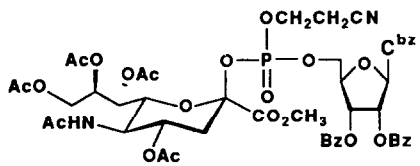


52

To instigate studies pertaining to the enzymatic transfer of unnatural sialic acid, Kondo *et al* [39] devised the synthesis of a protected CMP-sialic acid derivative (**54**) The approach entailed the preparation of the sialyl phosphoramidite **53** from sialic acid, its activation, and condensation with N^4-benzoyl-2',3'-di-*O*-benzoylcytidine After oxidation, **54** was obtained in 12% overall yield

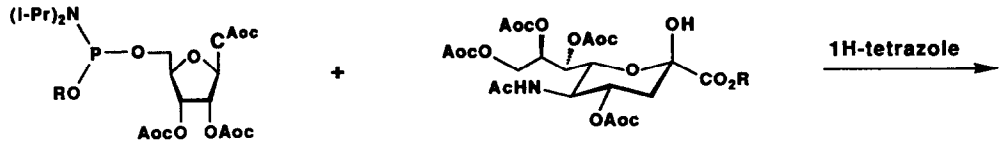


53

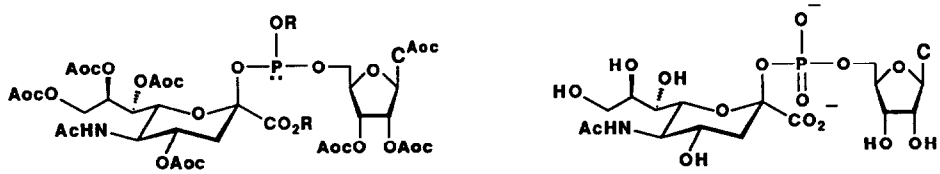


54

An alternate route to the chemical synthesis of cytidine 5'-monophosphono-*N*-acetylneuramic acid has been reported by Makino *et al* [40] Their method described the condensation of the ribonucleoside 5'-phosphoramidite **55** with the sialic acid derivative **56** Oxidation of **57** with *tert*-butyl hydroperoxide followed by removal of the protecting groups with triphenylphosphine and tetrakis(triphenylphosphine) palladium (0) produced **58** in 25% yield



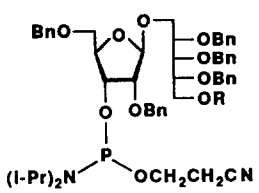
55 56



R= allyl 57 58

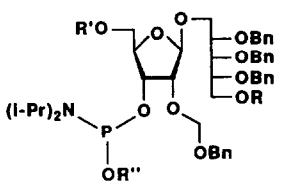
2 3. Phosphosugars

2 3 1 Poly-(ribosyl-ribitol)phosphates Phosphoramidite monomers have been applied to the preparation of the poly-(ribosyl-ribitol)phosphate (PRP) capsular polysaccharide isolated from *Haemophilus influenzae* Type b bacteria (*Hib*) There is considerable interest in PRP since it has been shown that covalent linking of an intact PRP molecule or a synthetic fragment of PRP to an immunogenic protein led to the production of a vaccine that immunized infants against *Hib* meningitis [41] The chemical synthesis of PRP fragments became attractive, as the correlation between immunogenicity and the size of these fragments would determine the most antigenic PRP fragment In this context, Chan and Just[42a,b] prepared the monomeric ribitol-ribosyl phosphoramidite **59** or **61** from properly protected D-ribose and D-ribitol precursors [42c]

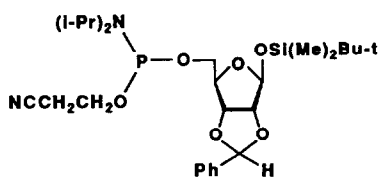


59 R= MMTr
60 = DMTr

Bn= benzyl; Ph= phenyl

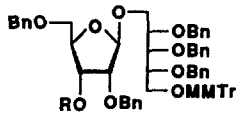


61 R= MMTr, R'= Si(Me)$_2$Bu-t; R"= Me
62 R= DMTr, R'= Si(Ph)$_2$Bu-t, R"= CH_2CH_2CN

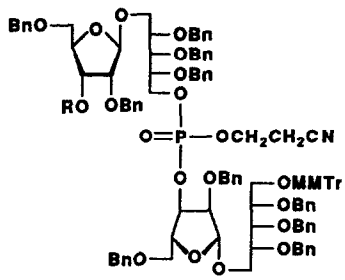


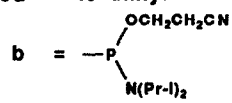
63

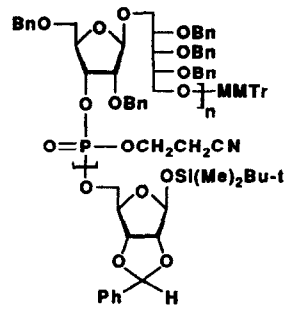
A PRP fragment was synthesized by coupling polysaccharide blocks together For example, the trisaccharide **66** was prepared from the reaction of activated phosphoramidite **63** with the ribitol-ribofuranoside **64a** followed by aqueous iodine oxidation [42b] Another polysaccharide block was similarly synthesized by condensation of the phosphoramidite **59** with detritylated **64b** The resulting tetrasaccharide (**65a**) was converted to the phosphoramidite **65b** and coupled with detritylated **66** to generate **67** Finally, the condensation of **65b** with detritylated **67** afforded, after oxidation, the PRP derivative **68** [42b]



64a R= H
b = levulinyl

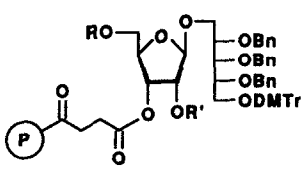


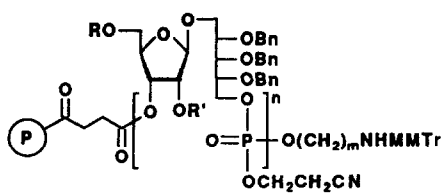
65a R= levulinyl
b = 



66 n= 1
67 = 3
68 = 5

A solid-phase synthesis of PRP fragments has been accomplished by Elie *et al.*[43] The D-ribitol phosphoramidite **62** was prepared, activated, and condensed repeatedly with support **69** The oligomeric chain was terminated with the coupling of 2-cyanoethoxy-[6-(4-monomethoxytrityl) aminohexyloxy]-*N,N*-diisopropylaminophosphine to afford the fully protected PRP fragment **71** The coupling efficiency of **62** was 96% Kandil *et al*[44] have virtually reproduced this synthetic approach using the soluble polymeric support **70** and the ribosyl-ribitol phosphoramidite **60** The coupling efficiency of **60** was greater than 90%. Termination of the synthesis with the condensation of an aminoheptyl phosphoramidite derivative led to the PRP oligomers **72** The conjugation of the larger PRP oligomers ($n \geq 3$) with proteins and synthetic peptides produced potent immunogens [44]





69 R= $Si(Ph)_2Bu$-t, R'= CH_2OBn
(P)= aminated CPG

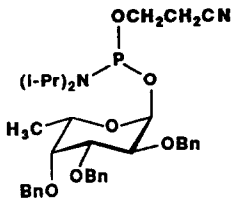
70 R= R'= Bn
(P)= PEG (M W. 5,000)

71 R= $Si(Ph)_2Bu$-t; R'= CH_2OBn
n= 6, m= 6
(P)= aminated CPG

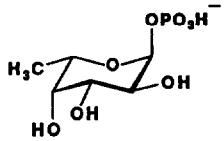
72 R= R'= Bn
n= 2,3,5,6; m= 7
(P)= PEG (M W 5,000)

2.3 2. Glycosyl phosphates The synthesis and application of the phosphoramidite **73** in the preparation of biologically important glycosyl phosphates have been delineated by Westerduin *et al*[45] Essentially, the activation of **73** with 1*H*-tetrazole in the presence of 3-hydroxypropionitrile yielded the bis-(2-cyanoethyl) phosphite triester which upon oxidation with *tert*-butyl hydroperoxide and complete deprotection afforded the α-L-fucopyranosyl phosphate **74** without detectable anomerization Furthermore, the reaction of the phosphoramidite **73** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranose under similar conditions generated the disaccharide phosphate **75** It must, however, be noted that the

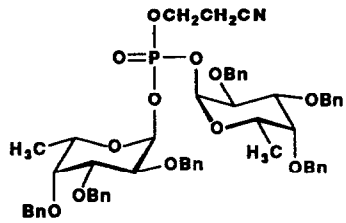
removal of the cyanoethyl phosphate protecting group from **75** by ammonolysis led to some cleavage of the phosphate linkage [45]



73



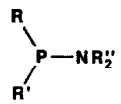
74



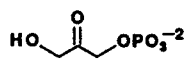
75

Of interest, bis-(benzyloxy)-*N,N*-diethylaminophosphine (**76c**) has been applied to the synthesis of an anomeric mixture of **74** toward the preparation of guanosine 5'-diphospho-ß-L-fucose (GDP-Fuc), a donor substrate for fucosyl-transferases [46] The phosphitylating reagent **76c** has alternatively been used in the preparation of ß-sialyl dibenzyl phosphite, an important intermediate in the synthesis of α-(2→6)- and α-(2→3)-linked sialyl saccharides [39] Sialylation is still considered a major problem in oligosaccharide synthesis

In a different context, **76c** enabled the chemical synthesis of dihydroxyacetone phosphate (**80**), which is required for the catalytic activity of at least three aldolases.[47] Enzymatic aldol reactions have been particularly useful in the synthesis of common and uncommon sugars [47]



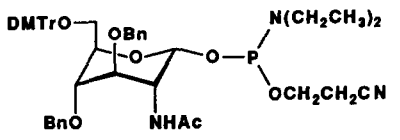
76a	R= R'= methoxy;	R''= ethyl	
b	= R'= ethoxy,	= ethyl	
c	= R'= benzyloxy,	= ethyl	
d	= R'= 2-cyanoethoxy,	= ethyl	
e	= R'= tert-butoxy,	= ethyl	
f	= R'= phenoxy;	= ethyl	
g	= R'= 4-bromobenzyloxy;	= ethyl	
77a	= R'= benzyloxy,	= isopropyl	
b	= R'= 2-cyanoethoxy,	= isopropyl	
c	= R'= allyloxy,	= isopropyl	
d	= R'= 4-chlorobenzyloxy,	= isopropyl	
e	= R'= tert-butoxy,	= isopropyl	
f	= R'= 2-trimethylsilylethoxy;	= isopropyl	
78	= benzyloxy,	R'= 2-cyanoethoxy;	R''= ethyl
79a	= benzyloxy;	= N,N-diethylamino,	= ethyl
b	= benzyloxy,	= N,N-diisopropylamino,	= isopropyl
c	= 2-trimethylsilylethoxy,	= N,N-diisopropylamino;	= isopropyl
d	= 2-cyanoethoxy,	= N,N-diisopropylamino;	= isopropyl
e	= allyloxy,	= N,N-diisopropylamino;	= isopropyl



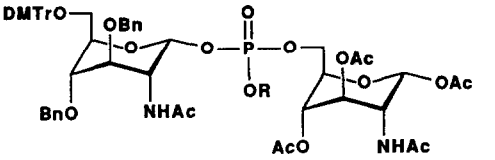
80

The phosphoramidite approach has been helpful in the synthesis of polymeric *N*-acetyl-D-glucosamine phosphates, which are important components of the cell wall of the bacteria *Micrococcus* sp 2102 (*Staphylococcus lactis*) [48] To achieve the formation of an α-(1→6)-interglycosidic phosphodiester linkage between two *N*-acetyl-D-glucosamines, the *N*-acetylglucosamine phosphoramidite **81** was synthesized and coupled with the 6-OH function of protected *N*-acetyl-α-D-glucosamine under standard conditions to produce the phosphosugar **82** [49a]

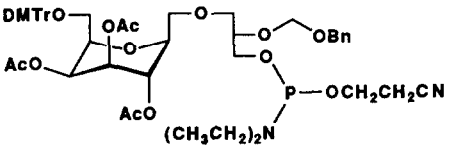
Aside from peptidoglycans, teichoic acid is a major component of the cell wall of most Gram-positive bacteria [48] Monomeric phosphoramidite derivatives have been effective in the synthesis of these immunologically and serologically important biopolymers Specifically, the 1-*O*-[ß-galacto-pyranosyl]glycerol phosphoramidite **83** has been prepared and utilized in the solid-phase synthesis of a teichoic acid fragment of the cell wall of *Bacillus licheniformis* ATCC 9945 [49b]



81

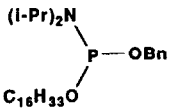


82 R= CH_2CH_2CN

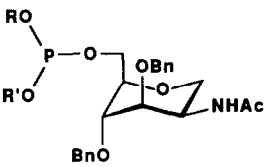


83

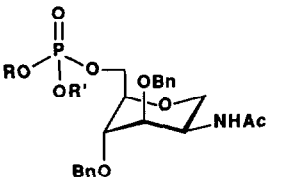
Broxterman *et al.*[50] reported the synthesis of 2-acetamido-2-deoxy-3-mannose analogues as potential inhibitors of 5-*N*-acetylneuraminic acid biosynthesis The phosphoramidite **84** and bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) have been employed in the preparation of the D-mannitol derivatives **85a-b** and **86a-b** It was postulated that, once deprotected, these mannosamine analogues could inhibit the enzyme-catalysed aldol condensation of *N*-acetyl-D-mannosamine-6-phosphate with phosphoenolpyruvate and, perhaps, alter specific biological recognition processes[50] The biological activity of such analogues was not reported



84

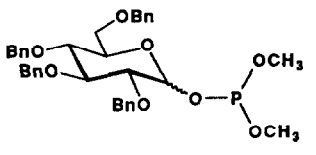


85a R= R'= Bn
b R= Bn, R'= $C_{16}H_{33}$

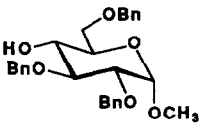


86a-b

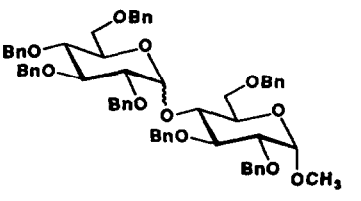
Particularly interesting is the reaction of 2,3,4,5-tetra-*O*-benzyl-α-D-glucose with bis-(methoxy)-*N,N*-diethylaminophosphine (**76a**) and 1*H*-tetrazole affording the glycosyl phosphite **87** as a mixture of anomers The condensation of **87** with the sugar **88** in the presence of zinc chloride and silver perchlorate gave **89** in 80% yield[51] 1-Glycosyl phosphites thus provide a new route to the synthesis of glycosides



87

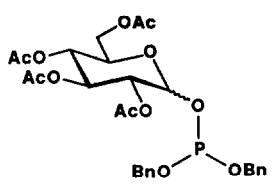


88

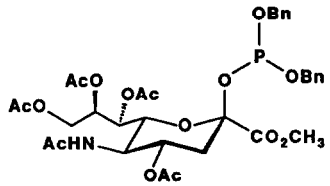


89

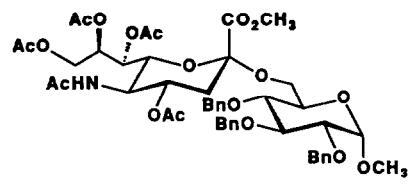
Along similar lines, the phosphitylation of 2,3,4,6-tetra-*O*-acetyl-D-glucose with **76c** and 1,2,4-triazole afforded the D-glucopyranosyl phosphite **90** in 97% yield [52] Oxidation of **90** with 30% hydrogen peroxide and full deprotection gave glucose 1-phosphate in 59% yield as a mixture of α- and ß-anomers The sialyl phosphite **91** has similarly been prepared from the corresponding sialyl alcohol, **76c**, and 1*H*-tetrazole. The phosphite **91** was either converted to its phosphate or treated with the glycosyl donor methyl glucopyranoside in the presence of trimethylsilyl triflate to generate the sialoside **92** [52]



90



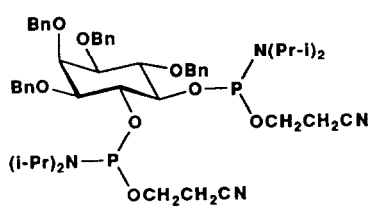
91



92

2 3 3 Myo-inositol phosphates Recent evidence suggests that the metabolism of inositol phospholipids produces at least two second messengers, one of which regulates the mobilization of calcium ion within stimulated cells [53a,c] It has been shown that D-*myo*-inositol 1,4,5-trisphosphate acts as the probable intracellular second messenger for calcium mobilization [53a-e] Related inositol phosphates, such as *myo*-inositol 1,3,4-trisphosphate[54a] and *myo*-inositol 1,3,4,5-tetrakisphosphate,[54b] have also been isolated from stimulated cells even though the biological roles of these species have not yet been fully understood To shed light into the mechanisms involved, the preparation of inositol phosphates and their analogues by chemical synthesis has been recommended [55,56a] In this context, the stereochemistry and nomenclature pertaining to these biomolecules have emerged from the recommendations of the International Union of Biochemistry [56b]

Phosphorylation of vicinal hydroxyl groups in *myo*-inositol can be difficult, as cyclic phosphates may form Pertinent to this problem, Hamblin *et al* [57] reported the preparation of *myo*-inositol 4,5-bisphosphate and its 4,5-bisphosphorothioate analogue Their synthetic approach involved the phosphitylation of DL-1,2,3,6-tetra-*O*-benzyl-*myo*-inositol with chloro-(2-cyanoethoxy)-*N,N*-diisopropyl-aminophosphine The resulting bis-phosphoramidite **93** was converted to the bis-phosphotriester **94a-b** upon treatment with 3-hydroxypropionitrile/1*H*-tetrazole followed by oxidation with either *tert*-butyl hydroperoxide[57b] or elemental sulfur [57a] Full deprotection of **94a-b** was effected by sodium in liquid ammonia, which led to the isolation of the DL-*myo*-inositol 4,5-bisphosphate and its 4,5-bis phosphorothioate (**95a-b**) in good yields [57]



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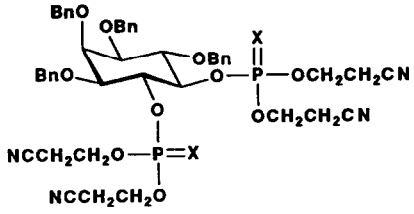
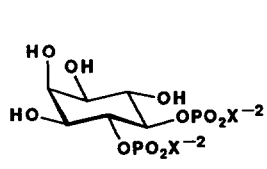
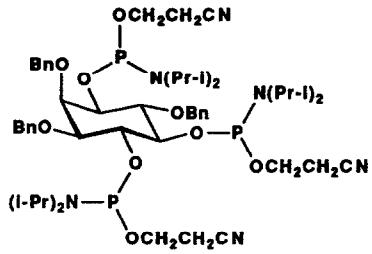


94a X= O
b = S



95a-b

DL-*Myo*-inositol 1,4,5-trisphosphate[58a-d,f,g] and its tristhiophosphate[58c-g] (**97a** and **97b**) were similarly prepared from the phosphoramidite **96** Like DL-*myo*-inositol 1,4-bisphosphorothioate,[59] **95b** and **97b** were expected to exhibit phosphatase resistance This feature is of biological significance since 5-phosphatase-catalysed breakdown of *myo*-inositol phosphates is an important process in second



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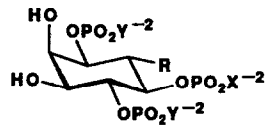
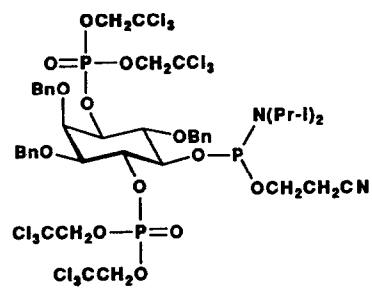


97a X= Y= O, R= OH
b = Y= S; = OH
c = S; Y= O, = OH
d = Y=O, = H

messenger deactivation and metabolism [59]

Cook *et al* [60a] and Noble *et al.*[60b] described the application of the *myo*-inositol phosphoramidite **98** to the chemical synthesis of the novel analogue DL-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate **97c** The phosphoramidite **98** was first prepared in several steps from DL-2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol [58f,g] Reaction of activated **98** with 3-hydroxypropionitrile afforded, after oxidation with *tert*-butyl hydroperoxide or elemental sulfur, **99a-b**. Deprotection of **99a-b** with sodium in liquid ammonia gave **97a** or **97c** in good yields.[60b] Interestingly, 2,2,2-trichloroethyl phosphate protecting groups were cleaved under these conditions.



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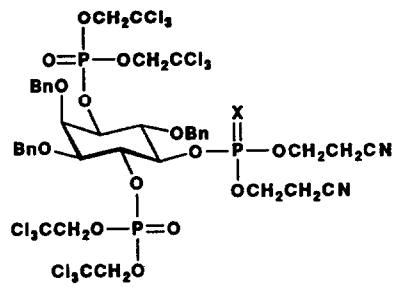
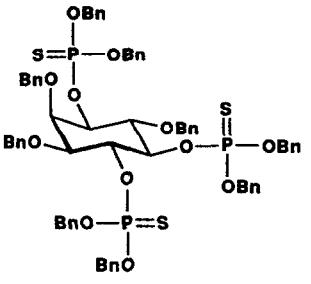


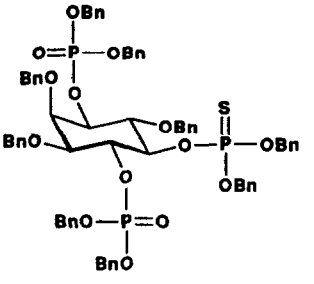
99a X= O
b = S

The preparation of racemic and chiral *myo*-inositol 1,4,5-trisphosphate derivatives from properly protected *myo*-inositols and bis-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphine (**77b**) has also been reported by Desai *et al* [61] Incidentally, the phosphitylation of racemic 2,3,6-tri-*O*-benzyl-*myo*-inositol with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) followed by sulfurization with phenacetyl disulfide produced 2,3,6-tri-*O*-benzyl-*myo*-inositol 1,4,5-tris-(dibenzylphosphorothioate) (**100**) in 88% yield. Removal of the benzyl protecting groups by reduction with sodium in liquid ammonia gave the sodium salt of **97b** in 51% yield [62] The synthesis of the *myo*-inositol phosphorothioates **101** and **102** was also reported

Cook *et al*,[63a] Strupish *et al.*,[63b] and Taylor *et al.*[63c] confirmed that DL-*myo*-inositol 1,4,5-trisphosphorothioate (**97b**) is a phosphatase-resistant analogue of *myo*-inositol 1,4,5-trisphosphate (**97a**) It has also been confirmed that **97b** mobilizes calcium from the intracellular stores of *Xenopus* oocytes,[63d] permeabilized hepatocytes,[63c] and Swiss 3T3 cells [63b,d] The calcium release activity of racemic **97b** is attributable to the D-isomer and was only *ca* 3-fold lower than that of racemic **97a** [63c] It must be noted that DL-*myo*-inositol 1,4-bisphosphate 5-phosphorothioate (**97c**) exhibited calcium release properties similar to those of DL-**97b** [63a] In addition, **97b** and **97c** were resistant to hydrolysis catalyzed by human erythrocyte 5-phosphatase and potently inhibited the enzyme [64a,b] While **97b** was



100



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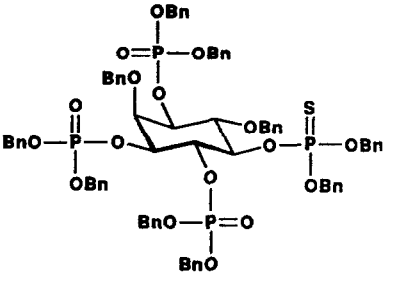
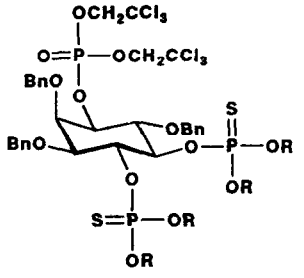


102

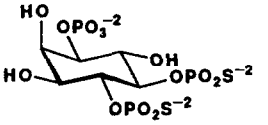
resistant to D-*myo*-inositol 1,4,5-trisphosphate 3-kinase, it would appear that **97c** underwent phosphorylation to inositol 1,3,4-trisphosphate 5-phosphorothioate under similar conditions.[64a]

Of interest, D-6-deoxy-*myo*-inositol 1,4,5-trisphosphate (**97d**), a synthetic analogue of the second messenger D-*myo*-inositol 1,4,5-trisphosphate mobilized intracellular calcium stores in permeabilized SH-SY5Y neuroblastoma cells but was 70-fold less potent than D-*myo*-inositol 1,4,5-trisphosphate in this respect.[65] These results indicate that the 6-hydroxy function of D-*myo*-inositol 1,4,5-trisphosphate is important for receptor binding and stimulation of calcium release but is not an essential structural feature. The *myo*-inositol analogue **97d** is not a substrate for *myo*-inositol 1,4,5-trisphosphate 5-phosphatase but appears to be a substrate for *myo*-inositol 1,4,5-trisphosphate 3-kinase.[65]

The elegant synthesis of *myo*-inositol 1-phosphate-4,5-pyrophosphate as a novel second messenger analogue from *myo*-inositol 1-phosphate-4,5-bisphosphorothioate has been reported by Noble *et al*.[66] Typically, DL-2,3,6-tri-*O*-benzyl-1-[bis-(2,2,2-trichloroethyl)phosphoryl]-*myo*-inositol, prepared from a properly protected inositol derivative, was phosphitylated with either bis-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphine (**77b**) or bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) and afforded **103a** or **103b** after oxidation with sulfur in pyridine. Deprotection of **103a** or **103b** with sodium in liquid ammonia followed by purification gave DL-**104** in 83% yield. This analogue was a potent agonist for intracellular calcium mobilisation in permeabilized SH-SY5Y neuroblastoma cells and resisted hydrolysis by D-*myo*-inositol 1,4,5-trisphosphate 5-phosphatase. Like *myo*-inositol 1,4,5-trisphosphorothioate, the bisphosphorothioate **104** stimulated a persistant release of calcium and potently inhibited D-*myo*-inositol 1,4,5-trisphosphate 5-phosphatase with a K_i of 1.3 ± 0.3 μM.[66] Desulfurization of **104** with *N*-bromosuccinimide[67] gave **105** in 67% yield. Phosphate migration (24%) and desulfurization to *myo*-inositol 1,4,5-trisphosphate (7%) were also observed. The *myo*-inositol pyrophosphate **105** was characterized by ^{31}P-NMR spectroscopy, and its biological properties are currently being evaluated.[66]



103a R= CH_2CH_2CN
b = Bn



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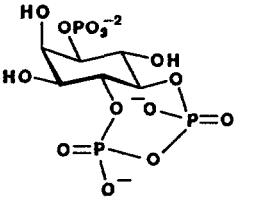
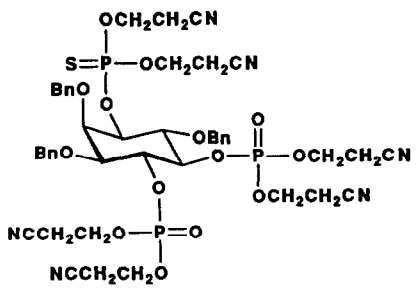
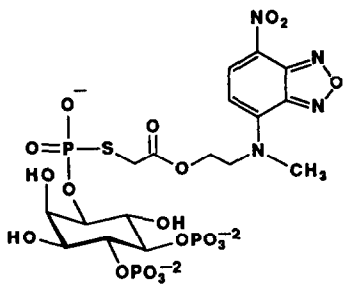


105

The fully protected inositol 1-phosphorothioate 4,5-bisphosphate **106** has been prepared from 1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol, bis-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphine (**77b**)/1*H*-tetrazole and proper oxidation/sulfurization reactions [68a-b] After full deprotection, the resulting *myo*-inositol 1-phosphorothioate 4,5-bisphosphate was coupled with *N*-[{2-(iodoacetoxy)ethyl}-*N*-methyl] amino-7-nitro-2,1,3-benzoxadiazole and generated the fluorescently labelled *myo*-inositol trisphosphate **107** This analogue strongly released ATP-sequestered intracellular calcium from permeabilized cells, thereby indicating its recognition by the *myo*-inositol 1,4,5-trisphosphate receptor [58f,68a-b] In view of this biological activity and fluorescence, the synthesis of the second messenger **107** should facilitate the study of its interactions with proteins It must be noted that DL-1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol was resolved into its enantiomers *via* crystalline 4,5-biscamphanate esters and that 1D(+)-1-*O*-allyl-2,3,6-tri-*O*-benzyl-*myo*-inositol was used in the preparation of D-*myo*-inositol-1-phosphorothioate 4,5-bisphosphate [68b]

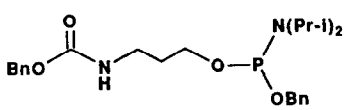


106

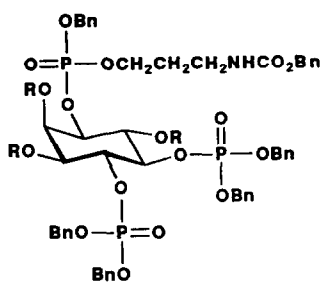


107

In this context, the phosphitylation of 2,3,6-tri-*O*-benzoyl-4,5-bis-*O*-(dibenzylphosphoryl)-D-*myo*-inositol with the phosphoramidite **108** and 1*H*-tetrazole afforded, after oxidation with MCPBA, the *myo*-inositol derivative **109a** in 74% isolated yield [69]

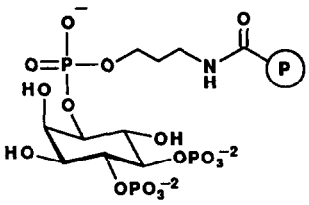


108



109a R= Bz
b = Bn

The myo-inositol **109a** was fully deprotected and combined with carbonyldiimidazole-activated agarose to yield **110**, which can be used as an affinity matrix for the isolation of D-*myo*-inositol 1,4,5-trisphosphate binding proteins [69] Deprotected **109a-b** was alternatively treated with the *N*-hydroxysuccinimide ester of 4-azido-2-hydroxybenzoic acid to give **111** This analogue exhibited good calcium ion-releasing activity, relative to D-*myo*-inositol 1,4,5-trisphosphate, in saponin-permeabilized rat basophilic leukemia cells and underwent light-induced cross-linking reaction with D-*myo*-inositol 1,4,5-trisphosphate receptor among other proteins [69]



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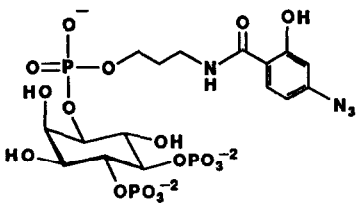


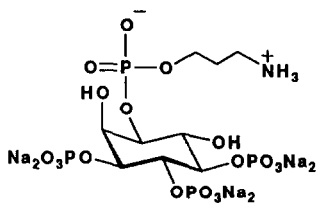
111

(P) = Agarose

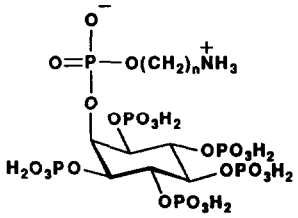
The *myo*-inositol **109b** was prepared by reaction of activated **108** with 2,3,6-tri-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-inositol followed by oxidation with MCPBA Ketal hydrolysis and phosphitylation of the resulting *myo*-inositol with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) yielded **109b** after oxidation [70a,h] Furthermore, phosphitylation of suitably protected *myo*-inositols with phosphoramidites **77a** and **108** generated the derivatives **112, 113a, 114** and **115** [70b-d,g,h]

Coupling **112** with a *N*-hydroxysuccinimide-activated resin allowed the binding of all *myo*-inositol 1,3,4,5-tetrakisphosphate and *myo*-inositol 1,2,3,4,5,6-hexakisphosphate receptors from partially purified and solubilized cerebellar membrane proteins [70b,e] Similar bioaffinity matrices have been prepared by use of **113a-b, 114** and **115** in an attempt to isolate putative binding proteins [70c-d] The reaction of **112** and **113a-b** with the *N*-hydroxysuccinimide ester of 4-azido-2-hydroxybenzoic acid provided the corresponding photoaffinity labels [70b-c,f]

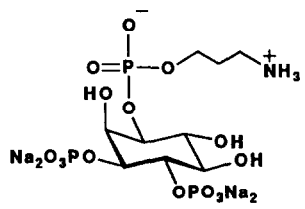
Various 2-substituted *myo*-inositol 1,4,5-trisphosphates for either photoaffinity labeling experiments or affinity chromatography have also been synthesized by Ozaki *et al* [71]



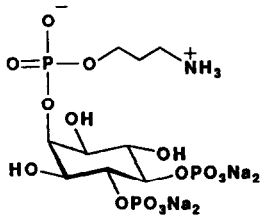
112



113a n= 3
b = 6



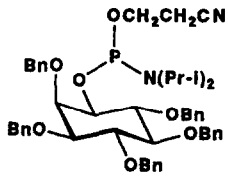
114



115

To investigate the kinetic and mechanistic properties of inositol monophosphatase, the synthesis of the bis-cyclohexylammonium salt of racemic *myo*-inositol 1-phosphorothioate (**118**) was undertaken This *myo*-inositol analogue was obtained from the deprotection of **117**, which was prepared from the

reaction of the *myo*-inositol phosphoramidite **116** with hydroxypropionitrile/1*H*-tetrazole and subsequent oxidation with elemental sulfur in pyridine Relative to inositol 1-phosphate, **118** was slowly converted to inositol by inositol monophosphatase.[72] It is also noteworthy that unlike the biosynthesis of L-*myo*-inositol 1-phosphate from D-glucopyranose 6-phosphate and inositol synthase, the synthesis of L-*myo*-inositol 1-phosphorothioate from D-glucopyranose-6-phosphorothioate failed under identical conditions D-Glucopyranose-6-phosphorothioate was not a substate for inositol synthase and thus stressed the need for an unmodified phosphate group for synthase function [72]



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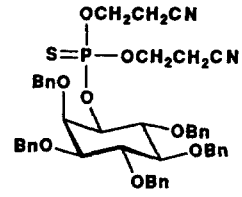
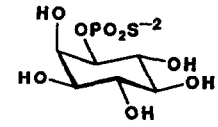
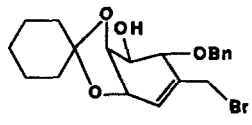


117

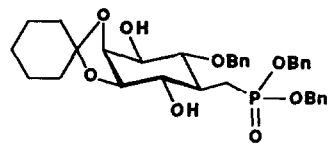


118

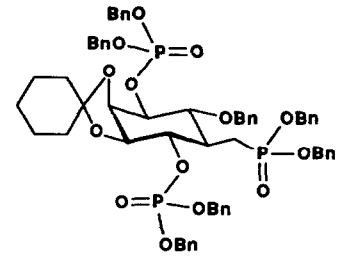
The total synthesis of the 5-methylenephosphonate analogue of D-*myo*-inositol 1,4,5-trisphosphate from (−)-quinic acid, has been reported by Falck *et al.* in an attempt to rationalize better the phosphatidylinositol cycle [73a,b] Schematically, the allylic bromide derivative **119** was treated with excess sodium dibenzyl phosphite and then hydroborated to the phosphoinositol **120** Phosphitylation of **120** with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) afforded **121** after MCPBA oxidation The sodium salt of the 5-methylenephosphonate analogue of D-*myo*-inositol 1,4,5-trisphosphate elicited contraction of bovine tracheal smooth muscle permeabilized with saponin and stimulated a sustained release of calcium from a microsomal preparation of bovine adrenal gland [73a,b] Incidentally, a chiral cyclitol has been prepared from (−)-quinic acid and employed in the synthesis of D-*myo*-inositol 3,4,5-trisphosphate and 1,3,4,5-tetrakisphosphate [73b,c]



119

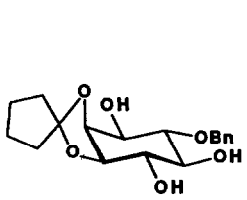


120



121

The preparation of *myo*-inositol 1,4,5-trisphosphate, according to the phosphoramidite approach, has independently been reported by Reese and Ward [74] Specifically, the phosphitylation of the *myo*-inositol derivative **122** with bis-(2-cyanoethoxy)-*N,N*-diethylaminophosphine (**76d**) generated **123** which, after oxidation with *tert*-butyl hydroperoxide and deprotection, gave the racemic *myo*-inositol 1,4,5-trisphosphate It was shown that enantiomerically pure D-*myo*-inositol 1,4,5-trisphosphate was more efficient at releasing calcium ions from permeabilised rat acinar cells than racemic *myo*-inositol 1,4,5-trisphosphate [74] The phosphitylation of 2,3,6-tri-*O*-benzyl-*myo*-inositol with **76d** has also led to the synthesis of *myo*-inositol 1,4,5-trisphosphate [75] Furthermore, bis-(2-cyanoethoxy)-*N,N*-diethylamino-phosphine has similarly been applied to the synthesis of D-*myo*-inositol 1,5-bisphosphate and 3,5-bisphosphate from optically resolved 2,3,4,6-tetra-*O*-benzyl-*myo*-inositol [76]



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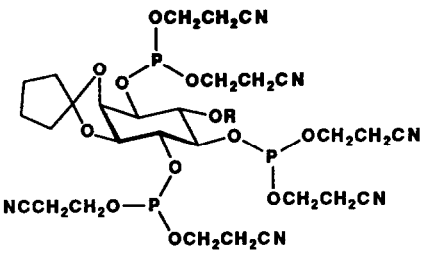
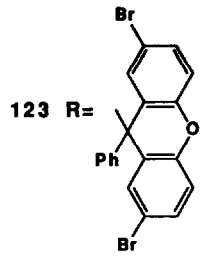
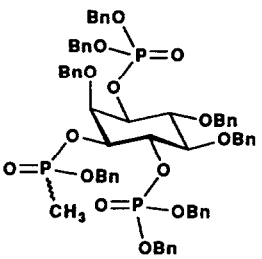


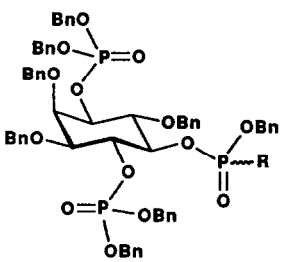


123 R=

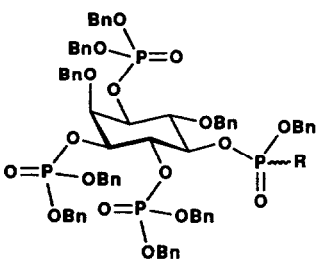
Several researchers have additionally employed either bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) [77-82,84] or bis-(benzyloxy)-*N,N*-diethylaminophosphine (**76c**)[78,83] for the 1*H*-tetrazole-mediated phosphitylation of suitably protected inositols. After oxidation and deprotection, *myo*-inositol 1,3,4-trisphosphate,[78,81b] 1,4,5-trisphosphate,[77a,78-81a,83] 2,4,5-trisphosphate,[77b] and 1,3,4,5-tetrakisphosphate[78,81b-83] were obtained as racemic mixtures[78] or, in certain cases, in enantiomerically pure form [77,79-83] In other cases, the tetrasodium salts of 2,5-di-*O*-benzyl-*myo*-inositol 1,3,4,6-tetrakis-(benzyl phosphate) and D-2,6-di-*O*-benzyl-*myo*-inositol 1,3,4,5-tetrakis-(benzyl phosphate) were only isolated [84b] Furthermore, **77a** has been applied to the synthesis of the racemic 3-methylphosphonate analogue of *myo*-inositol 1,3,4-trisphosphate (**124**)[85] along with the 5-phosphonate analogues of *myo*-inositol 1,4,5-trisphosphate (**125a-b**)[86,87] and 1,3,4,5-tetrakisphosphate (**126a-b**).[87] These analogues may provide structural information regarding the biological pathways involved with the mechanism of cellular signal transduction [85] In fact, the 5-methylphosphonate analogue of *myo*-inositol 1,4,5-trisphosphate acted as a calcium antagonist in permeabilized human platelets [87]



124



125a R= Me
b = CHF_2

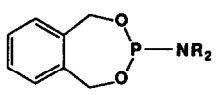


126a R= Me
b = CHF_2

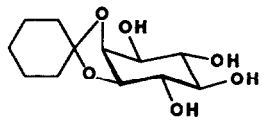
Watanabe *et al.*[88a] described the efficient phosphitylation of 2,3,6-tri-*O*-benzyl-*myo*-inositol with 2-(*N,N*-diethylamino)-5,6-benzo-1,3,2-dioxaphosphepane (**127a**) and 1*H*-tetrazole Following oxidation with MCPBA, the phosphorylated *myo*-inositol was converted to *myo*-inositol 1,4,5-trisphosphate in 97% yield upon hydrogenolysis Substituting elemental sulfur for MCPBA generated the *myo*-inositol 1,4,5-tristhiophosphate **97b** in 81% yield It has been pointed out that the phosphoramidite **127a** was easier to purify than bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) or bis-(benzyloxy)-*N,N*-diethylaminophosphine (**76c**) [88a]

An elegant and efficient resolution of racemic 2,3-mono-*O*-cyclohexylidene-*myo*-inositol by enzymatic esterification in organic solvents has been reported by Ling and Ozaki [89] Thus, the reaction of racemic **128** with acetic anhydride in the presence of Lipase CES (*Pseudomonas sp*) led to exclusive acetylation of the L-enantiomer at *C*-1 The unreacted D-enantiomer was easily separated from the acetylated L-enantiomer by silica gel chromatography Limited acetylation of D-**128** with acetic anhydride afforded the 5- and 6-monoacetylated D-*myo*-inositol derivatives **129** and **130** (74% yield) in

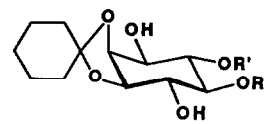
a 1 1 ratio Phosphitylation of **130** with **127a** eventually produced, after oxidation and deprotection, D-*myo*-inositol 1,4,5-trisphosphate in an overall yield of *ca.* 13% based on racemic *myo*-inositol [89] The synthesis of D-*myo*-inositol 1,4,5-trisphosphate has also been achieved by Ozaki *et al* [90] using **127a** and adequately protected D-*myo*-inositol



127a R= ethyl
b = methyl

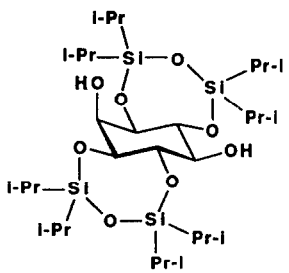


128

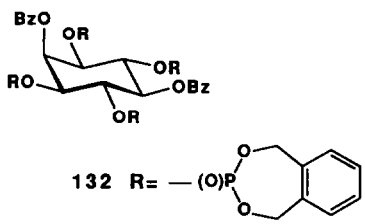


129 R= Ac; R'= H
130 = H; = Ac

A practical synthesis of *myo*-inositol 1,3,4,5-tetrakisphosphate has been accomplished by reaction of *myo*-inositol with limited amounts of benzoyl chloride at 90 °C [88b,e,f] In a specific case, the resulting *myo*-inositol 1,3,4,5-tetrabenzoate was benzylated and then debenzoylated to the corresponding 1,3,4,5-tetrol, which was phosphitylated with **127a** After oxidation and hydrogenolysis, the desired *myo*-inositol 1,3,4,5-tetrakisphosphate was isolated in high yields [88b,91] In the same context, *myo*-inositol 1,3,4,6-tetrakisphosphate has been conveniently prepared from the bis-(disiloxane) **131**, which was obtained from the regioselective protection of *myo*-inositol with 1,3-dichloro-1,1,3,3-tetraisopropyl disiloxane [88c,91] Benzoylation of **131**, followed by treatment with aqueous hydrogen fluoride in acetonitrile, produced the *myo*-inositol 2,5-dibenzoate in *ca.* 96% yield Phosphitylation of the latter compound with **127a** and subsequent oxidation with MCPBA generated the tetraphosphotriester **132** in 94% yield Removal of the protecting groups by hydrogenolysis and ammonolysis gave *myo*-inositol 1,3,4,6-tetrakisphosphate in 80% yield [88c]



131



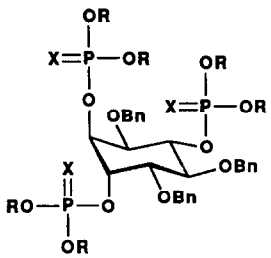
132 R= —(O)P

The phosphoramidite **127a** has alternatively been applied to the chiral synthesis of D-*myo*-inositol 1-phosphate from L-quebrachitol,[92] while **127b** was analogously used in the synthesis of D-*myo*-inositol 1,4,5-trisphosphate, 1,4-bisphosphate, and 4-phosphate *via* *myo*-inositol D-camphor-2,3-monoacetal precursors [93]

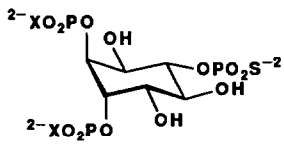
L-*chiro*-inositol 1,4,6-trisphosphate and trisphosphorothioate (**133a-b**) have also been synthesized from L-quebrachitol Specifically, L-*chiro*-2,3,5-tri-*O*-benzyl inositol, obtained from the demethylation and tin-mediated benzylation of L-quebrachitol, was phosphitylated with bis-(2-cyanoethoxy)-*N,N*-diisopropylaminophosphine (**77b**) Oxidation of the trisphosphite with either *tert*-butyl hydroperoxide or sulfur in pyridine afforded the *chiro*-inositol **133a** or **133b**, which after deprotection gave **134a** or **134b** [94]

Neither **134a** nor **134b** mobilized calcium or antagonized calcium mobilisation induced by *myo*-inositol 1,4,5-trisphosphate at concentrations up to 30 μM However, L-*chiro*-inositol 1,4,6-trisphosphorothioate (**134b**) competitively inhibited D-*myo*-inositol 1,4,5-trisphosphate 5-phosphatase

with a K_i of 0.3 μM [94] Thus, **134b** is by far the most potent and selective D-*myo*-inositol 1,4,5-trisphosphate 5-phosphatase inhibitor yet encountered The mechanism of this inhibition is still not clearly understood



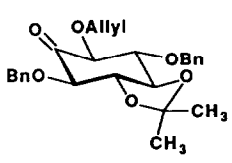
133a X= O, R= CH_2CH_2CN
b = S; = CH_2CH_2CN



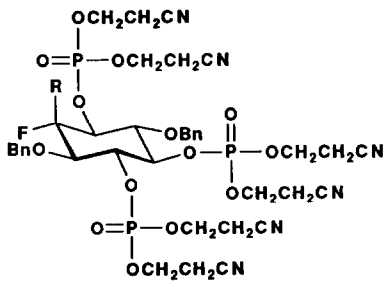
134a X= O
b = S

Syntheses of fluorinated analogues of inositol and inositol 1,4,5-trisphosphate have been reported [95a-c] For example, DL-2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate (**138**) was synthesized by reaction of 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-*myo*-2-ionose (**135**) with diethylaminosulfur trifluoride Subsequent deblocking of non-benzylic protecting groups followed by phosphitylation with **77b** and oxidation with *tert*-butyl hydroperoxide yielded the fluorinated inositol **136** Deprotection of the *myo*-inositol analogue by treatment with sodium in liquid ammonia gave DL-**138** [95a-c] Alternatively, the preparation of the (−)-camphanate ester of DL-3,6-di-*O*-benzyl-2-deoxy-2,2-difluoro-4,5-*O*-isopropylidene-*myo*-inositol enabled the chromatographic separation of the resulting diastereoisomers After cleavage of the camphanate and ketal functions, the synthesis of both D- and L-enantiomers of 2-deoxy-2,2-difluoro-*myo*-inositol 1,4,5-trisphosphate was achieved [95a-b]

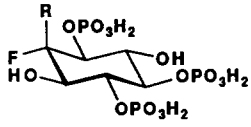
In this context, the synthesis of DL-2-deoxy-2-fluoro-*scyllo*-inositol 1,4,5-trisphosphate (**139**) began with the fluorination of racemic-3,6-di-*O*-benzyl-4,5-*O*-isopropylidene-1-*O*-[(*Z*)-prop-1-enyl]-*myo*-inositol (**140**) with diethylaminosulfur trifluoride The fluorinated inositol analogues **139** was then isolated in a manner similar to that described for **138** [95a] DL-2-deoxy-2-fluoro-*scyllo*-inositol 1,4,5-trisphosphate and DL-2,2-difluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate mobilized calcium but were slightly less potent than was D-*myo*-inositol 1,4,5-trisphosphate These results indicate that the axial 2-hydroxyl group of D-*myo*-inositol 1,4,5-trisphosphate is relatively unimportant in receptor binding and stimulation of calcium release [95c] It must be noted that while D-**138** is a potent calcium releasing agonist, L-**138** is a powerful 5-phosphatase and 3-kinase inhibitor [95a-b]



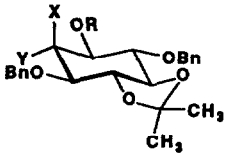
135



136 R= F
137 = H

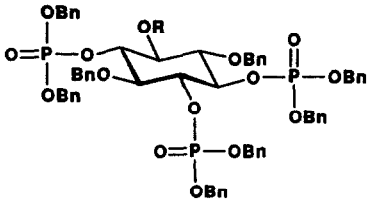


138 R= F
139 = H

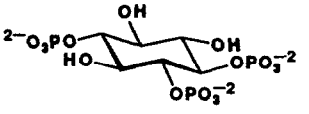


140	**X= OH,**	**Y= H;**	**R= (*Z*)-prop-1-enyl**
141	**= OH;**	**= H;**	**= allyl**
142	**= H;**	**= OAc;**	**= allyl**
143	**= H,**	**= OH,**	**= (*Z*)-prop-1-enyl**
144	**= F;**	**= H;**	**= (*Z*)-prop-1-enyl**

Lampe and Potter[96] recently synthesized *scyllo*-inositol 1,2,4-trisphosphate (**146**) and 2-fluoro-2-deoxy-*myo*-inositol 1,4,5-trisphosphate (**148**) as novel analogues of the second messenger *myo*-inositol 1,4,5-trisphosphate Essentially, racemic 1-*O*-allyl-3,6-di-*O*-benzyl-4,5-isopropylidene *myo*-inositol (**141**) was triflated and treated with cesium acetate to produce **142** Saponification of the acetate function and cleavage of the isopropylidene group followed by phosphitylation with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) and oxidation, produced the fully protected *myo*-inositol trisphosphate **145** Treatment of **145** with sodium in liquid ammonia removed all protecting groups including allyl and, thereby, afforded **146**[96] The saponification of **142** and isomerization of the allyl group led to **143** Conversion of **143** to the corresponding triflate and treatment with tetra-*n*-butylammonium fluoride generated **144** In a manner similar to that described for the preparation of **145**, the cleavage of the isopropylidene and prop-1-enyl groups allowed the synthesis of **147** which, after deprotection, gave **148**. Both racemic **146** and **148** exhibited calcium mobilizing properties similar to those of *myo*-inositol 1,4,5-trisphosphate, in permeabilized SH-SY5Y neuroblastoma cells.[96] These analogues should therefore provide a better understanding of the molecular recognition of *myo*-inositol 1,4,5-trisphosphate by binding proteins



145 R= allyl



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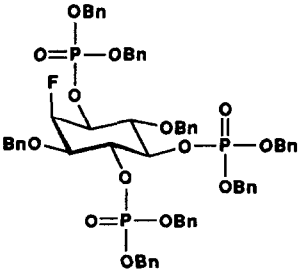

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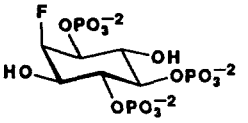
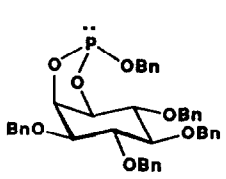


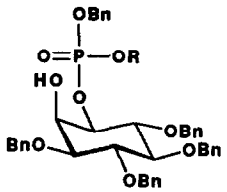
148

A regioselective synthesis of inositol phosphate diesters *via* cyclic phosphate triester intermediates has been reported by Schultz *et al*[97] Thus, **149** was prepared by condensation of 3,4,5,6-tetra-*O*-benzyl-*myo*-inositol with benzyloxy-bis-(*N,N*-diethylamino)phosphine (**79a**) and 1*H*-tetrazole Oxidation of **149** with MCPBA and reaction of the resulting cyclic phosphate with various alcohols led to the *myo*-inositol phosphotriesters **150a-e** and **151a-d** Low temperature and bulky alcohols gave higher selectivity for

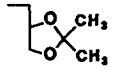
myo-inositol 1-phosphotriesters, whereas addition of sodium sulfite to a methanolic reaction mixture favored formation of the 2-phosphotriester **151a**.[97]



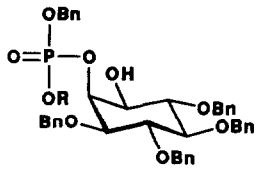
149



150a R= methyl
b = ethyl
c = isopropyl
d =

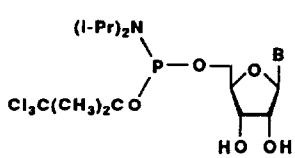


e = tert-butyl



151a-d

(3'→5')-Cyclic adenosine monophosphate (cAMP), an ubiquitous second messenger formed by transmembrane signalling systems, is required for the activity of a large number of hormones. The effects of cAMP on metabolic pathways are mediated by cAMP-dependent protein kinase through phosphorylation of various regulatory enzymes.[98] The phosphoramidite approach has been useful in the synthesis of cAMP derivatives. For example, Strasser and Ugi[99] reported the highly selective 5'-phosphitylation of N^6,N^6-bis-[(2,2,2-trichloro-*tert*-butyl)oxycarbonyl]adenosine with chloro-(2,2,2-trichloro-*tert*-butyloxy)-*N*,*N*-diisopropylaminophosphine in *N*,*N*-dimethylformamide at −30 °C. The ribonucleoside 5'-phosphoramidite **152** was formed in 93% yield whereas 3'- and 2'-phosphitylated ribonucleosides were generated in yields of only 4% and 3%, respectively. Activation of **152** with 5-(4-nitrophenyl)tetrazole promoted the quantitative formation of the cyclophosphite **153** as a mixture of stereoisomers. Oxidation of **153** with 3-(2,4-dichlorophenyl)-2-tosyloxaziridine produced the corresponding cyclophosphate **154**.[99] This approach appears particularly suited for the synthesis of various cAMP analogues.



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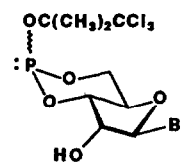

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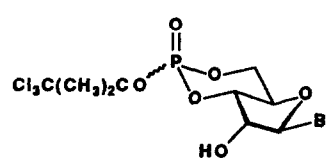
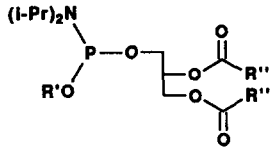


154

B= [N^6,N^6-di-(2,2,2-trichloro-tert-butyl)oxycarbonyl]adenin-9-yl

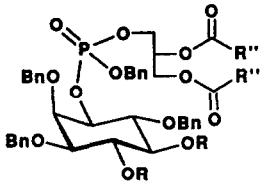
2 3 4 Myo-inositol phospholipids In their synthesis of 1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho)-D-*myo*-inositol-4,5-bisphosphate (**158**), Dreef *et al*.[100a] phosphitylated 1,2-di-*O*-palmitoyl-*sn*-glycerol with bis-(*N*,*N*-diisopropylamino)benzyloxyphosphine (**79b**) to yield the glycerophosphoramidite **155**. Coupling **155** with a properly protected *myo*-inositol gave **157a** after oxidation. Upon removal of the allyl groups, the free hydroxy functions were phosphitylated with bis-(benzyloxy)-*N*,*N*-diisopropylaminophosphine (**77a**). Oxidation and subsequent deprotection led to **158**.[100a] The glycerophosphoramidite derivative **155** has analogously been utilized in the synthesis of 1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphoryl)-2-*O*-α-D-mannopyranosyl-D-*myo*-inositol, a component of myco-

bacterial phospholipids [100b] It must be pointed out that both 2-(*N,N*-diethylamino)-5,6-benzo-1,3,2-dioxaphosphepane (**127a**)[88d] and diphenyl *N,N*-diethylphosphoramidite[101] have also been used in the synthesis of *myo*-inositol phospholipids



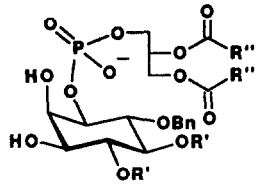
155 R'= Bn, R''= $C_{15}H_{31}$

156 R'= Me; R''= $C_{15}H_{31}$



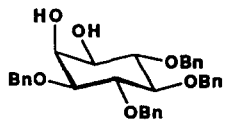
157a R= Allyl; R''= $C_{15}H_{31}$

b R= $P(O)OBn_2$; R''= $C_{15}H_{31}$



158 R'= PO_3^{-2} R''= $C_{15}H_{31}$

A convenient synthesis of phosphatidyl *myo*-inositol derivatives has additionally been reported by Watanabe *et al* [102] The reaction of the tetrabenzyl-*myo*-inositol **159** with the glyceryl phosphite **160** and pyridinium bromide perbromide in pyridine afforded **161** with excellent regioselectivity This approach has similarly been applied to the synthesis of protected derivatives of *myo*-inositol 1-phosphate and *myo*-inositol 1,4,5-trisphosphate [102]



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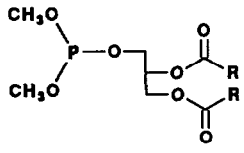
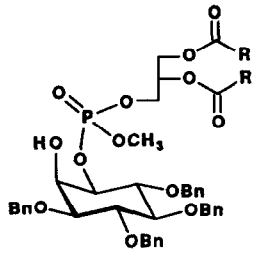
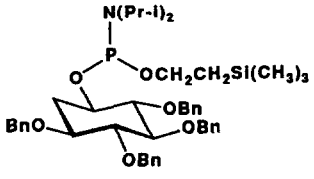


160 R= $C_{17}H_{35}$



161 R= $C_{17}H_{35}$

To further study the hydrolysis of phosphatidylinositides by phospholipase C, Seitz *et al* [103] synthesized a phosphatidylinositol analogue lacking the axial 2-hydroxyl group of the inositol moiety The D-2-deoxy-*myo*-inositol phosphoramidite **162** was generated from D-2-deoxy-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol and bis-(*N,N*-diisopropylamino)-2-trimethylsilylethoxyphosphine (**79c**) in the presence of 1*H*-tetrazole The reaction of activated **162** with dipalmitoyl glycerol followed by oxidation with dilute



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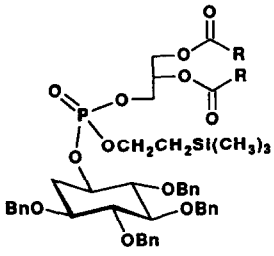
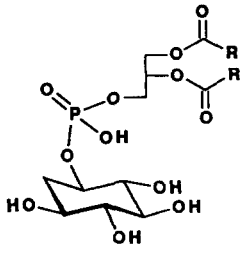


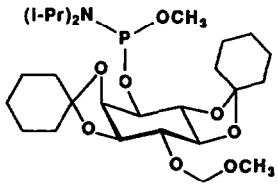
163 R= $C_{15}H_{31}$



164 R= $C_{15}H_{31}$

hydrogen peroxide afforded **163** which, after deprotection, gave **164** The phosphatidylinositol analogue **164** was not a substrate for phospholipase C, isolated from a human melanoma cell line, at concentrations up to 1 0 mM but was a weak inhibitor of the enzyme [103] These results are consistent with the hypothesis advocating a ribonuclease-like mechanism taking place during phospholipase C-catalyzed hydrolysis of phosphatidylinositides.

Racemic 4-(1-pyrenyl)butylphosphoryl-1-*myo*-inositol has been synthesized by reaction of the protected *myo*-inositol phosphoramidite **165** with 1-pyrenebutanol After oxidation with tetra-*n*-butylammonium periodate, **166** was isolated in *ca* 90% yield and was deprotected to **167** [104] The latter compound was a good substrate for phosphatidylinositol-specific phospholipase C and provided a very sensitive assay to measure the activity of the enzyme in crude preparations The detection limit of 1-pyrenebutanol was estimated to be 100 picomoles [104]



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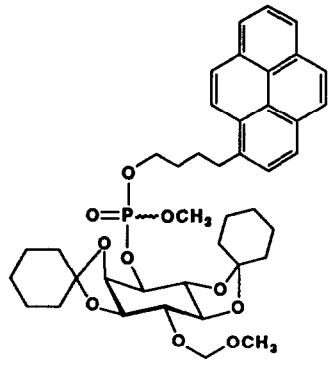

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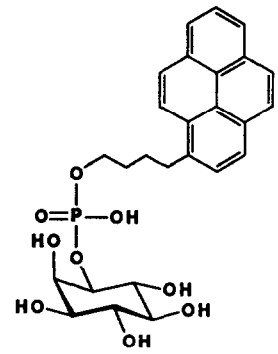
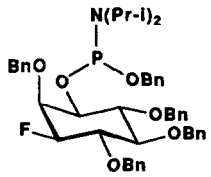


167

Of interest, the D-3-deoxy-3-fluorophosphatidylinositol phosphoramidite **168** has been prepared from D-3-deoxy-3-fluoro-*myo*-inositol in a multistep synthesis [105] Condensing **168** with 1,2-dipalmitoyl-*sn*-glycerol followed by oxidation gave the phosphatidyl inositol **169** and, after deprotection, **170** D-3-deoxy-3-fluorophosphatidylinositol (**170**) was 10-70 times more active than D-3-deoxy-3-fluoro-*myo*-inositol in inhibiting cell growth (NIH 3T3 and v-*sis* NIH 3T3 cells) [105] Thus, **170** may provide new alternatives toward the discovery of non-DNA targeted anti-cancer agents



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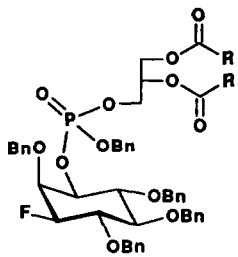
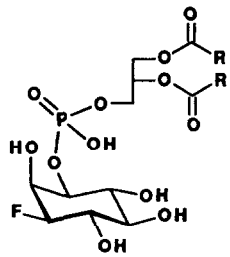


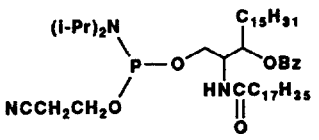
169 R= $C_{15}H_{31}$



170 R= $C_{15}H_{31}$

The total synthesis of the naturally occurring ceramide phosphoinositol has received some attention, as this phosphoinositide, among others, is believed to protect plant tissues from necrotic lesions and has been found in the complex structure of *Trypanosoma cruzi* lipopeptido-phosphoglycans [106] The racemic phosphoramidite **171** was prepared from the reaction of *rac*-3-

benzoylceramide with (2-cyanoethyl)-*N,N,N',N'*-tetraisopropylphosphorodiamidite (**79d**) and *N,N*-diisopropylammonium tetrazolide. The condensation of **171** with racemic 1,2,4,5,6-pentaacetyl-*myo*-inositol afforded, after oxidation, the ceramide phosphotriester **172** in 80-90% yield Deprotection of **172** produced the crystalline ceramide phospho-*myo*-inositol **173**.[106]



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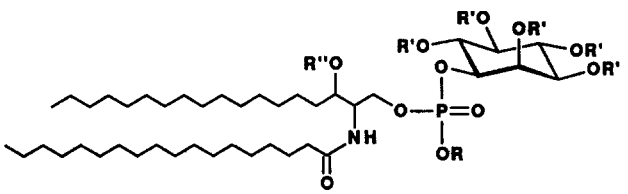
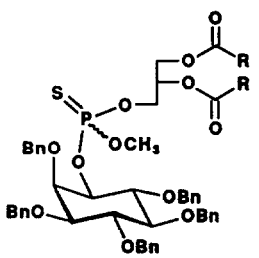
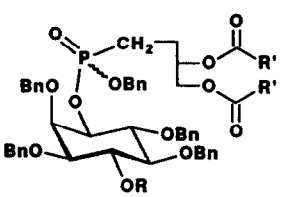


172 R= CH_2CH_2CN; R'= Ac; R"=Bz
173 R= R'= R"= H

The synthesis of the 1,2-dipalmitoyl-*sn*-glycero-3-thiophospho-1'-inositol derivative **174** has been achieved by reaction of the glycerophosphoramidite **156** with D-(-)-2,3,4,5,6-pentabenzyl-*myo*-inositol followed by sulfurization with elemental sulfur.[107a,b] The deprotected phosphorothioate analogue of dipalmitoyl phosphatidylinositol may serve as an antimetabolite blocking receptor-mediated inositol phosphate metabolism.[107a]



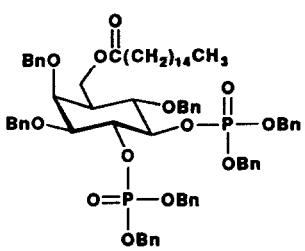
174 R= $C_{15}H_{31}$



175 R= H; R'= $C_{15}H_{31}$
176 = $P(O)OBn_2$; = $C_{15}H_{31}$

The activation of bis(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) with 1*H*-tetrazole enabled the phosphitylation of the D-*myo*-inositol phosphonate **175** which, after oxidation, provided the fully protected *myo*-inositol phospholipid analogue **176**.[108] D-*Myo*-inositol phosphonolipid can be useful in the detailed study of phospholipase C inhibition.

In the same context, **77a** has been used in the multistep synthesis of DL-(hexadecanoyloxy)methyl- and 1-*O*-hexadecanoyl-inositol derivatives (**177-179**).[109a-b] These analogues exhibited significant



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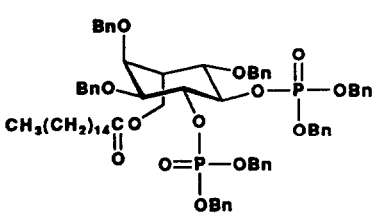

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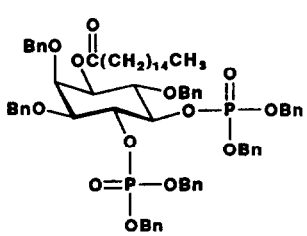
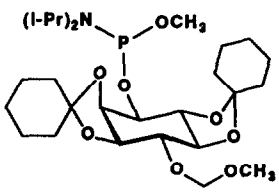


179

inhibition of phospholipase C *in vitro* However, none of these derivatives was able to inhibit phospholipase C in intact cells, presumably, because of poor cell penetration.[109b]

In an attempt to elucidate the steric course of the reaction catalyzed by phosphatidylinositide-specific phospholipase C from *Bacillus cereus* and guinea pig uterus, the *myo*-inositol phosphoramidite **180** has been applied to the preparation of the 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphoinositol derivative **181** from 1,2-dipalmitoyl-*sn*-glycerol [110a,b,d] It was found that the *Rp* isomer of 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphoinositol was the preferred substrate for all of the phosphatidylinositide-specific phospholipase C investigated. The conversion of the substrate to inositol 1,2-cyclic phosphorothioate and inositol phosphorothioate proceeded with inversion of configuration at phosphorus, *via* direct attack by the 2-OH group, without involving a covalent enzyme-phosphoinositol intermediate.



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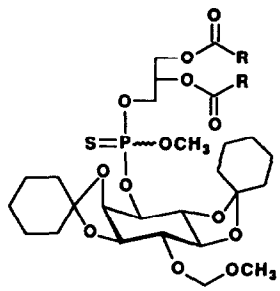
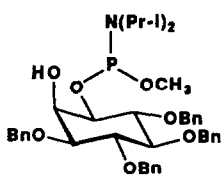


181 R= $C_{15}H_{31}$

The formation of inositol 1,2-cyclic phosphorothioate was confirmed by its independent chemical synthesis. Thus, the *myo*-inositol cyclic phosphorothioate **184** was generated, as a mixture of *cis* (*endo*) and *trans* (*exo*) isomers, from the intramolecular cyclization of the *myo*-inositol phosphoramidites **182** and **183** upon activation and sulfurization [110a,b,d]



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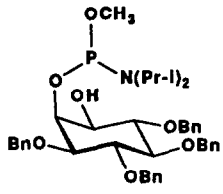

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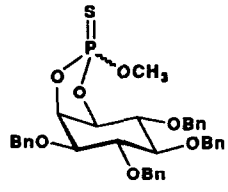
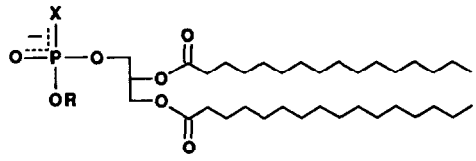


184

Bruzik *et al* [110c] subsequently demonstrated that phosphatidylinositols, chirally labeled at phosphorus, were converted to inositol 1,2-cyclic phosphate and inositol 1-phosphate by phosphatidylinositol-specific phospholipase C from *Bacillus cereus* with overall inversion and retention of configuration at phosphorus, respectively A sequential mechanism involving inositol 1-phosphate has been postulated [110c]

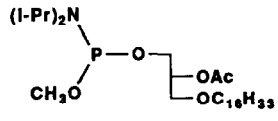
2 4 Phospholipids and Phospholipid Conjugates

To simplify the synthesis of phospholipids, the glycerophosphoramidite **156**, prepared from 1,2-dipalmitoyl-*sn*-glycerol and chloro-(*N,N*-diisopropylamino)methoxyphosphine, was treated with either choline tosylate, *N*-tritylethanolamine or 1,2-isopropylidene-*sn*-glycerol to give the corresponding glycerophosphite triesters Oxidation of these derivatives with *tert*-butyl hydroperoxide or elemental sulfur led, after deprotection, to the glycerophosphodiesters **185a-c** or the glycerophosphorothioates **186a-c** [107b,111]



185a R= $CH_2CH_2\overset{+}{N}(CH_3)_3$; X= O
b = $CH_2CH_2\overset{+}{N}H_3$
c = $CH_2CH(OH)CH_2OH$
186a-c X= S

The glycerophosphorothioate **188** was similarly prepared by condensation of the glycerophosphoramidite **187** with choline tosylate followed by sulfurization with elemental sulfur.[112] It has been shown that the *Sp* isomer of 1-*O*-hexadecyl-2-acetyl-3-thiophosphocholine (**188**) had the same activity in platelet aggregation and serotonin secretion as 1-*O*-hexadecyl-2-acetyl-3-phosphocholine (AGEPC) The *Rp* isomer of **188**, unlike the *Sp* isomer, was only 0 6-2% as active as AGEPC under identical conditions These findings suggest that the phosphate group of AGEPC is likely to interact with its receptor, at least in events leading to platelet aggregation and serotonin secretion.[113]



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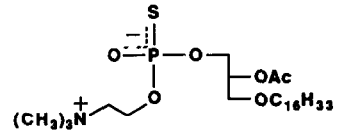
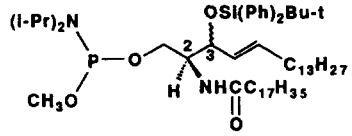
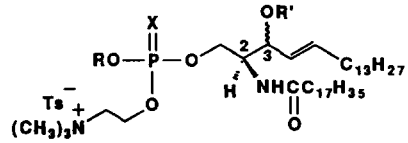


188

Although sphingomyelin is one of the most abundant component of biological membranes and blood plasma lipoproteins, its specific function in membranes is not well understood It has nevertheless been suggested that sphingomyelin formed stable complexes with cholesterol and strong intermolecular hydrogen bonds with other phospholipids [114a] To facilitate the biophysical and biochemical studies of these biomolecules, the chemical synthesis of sphingomyelin and its analogues has been carried out by Bruzik [114a,b] Typically, the *N*-stearoylsphingoside phosphoramidites **189a-b** were prepared by phosphitylation of D-*erythro*- and L-*threo*-2-*N*-stearoylsphingosine with chloro-(*N,N*-diisopropylamino)methoxyphosphine The reaction of **189a-b** with choline tosylate and 1*H*-tetrazole produced, after oxidation (sulfurization), the corresponding phosphotriesters **190a-b** and **191a-b** These derivatives were demethylated with anhydrous trimethylamine and desilylated with tetra-*n*-butylammonium fluoride to give the sphingomyelins **192a-b** and **193a-b** in isolated yields exceeding 75% [114]



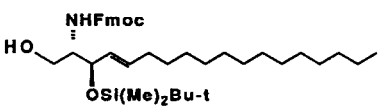
189a D-*erythro* (2*S*,3*R*)
b L-*threo* (2*S*,3*S*)
Ts^- = 4-toluenesulfonate



190a-b R= Me; R'= $Si(Ph)_2Bu$-t, X= O
191a-b = Me, = $Si(Ph)_2Bu$-t, = S
192a-b = R'= H, X= O
193a-b = R'= H; = S

There is evidence to suggest that sphingosine-1-phosphate (**196**) induces a rapid and sustained release of calcium from *myo*-inositol 1,4,5-trisphosphate-sensitive and insensitive intercellular pools in permeabilized smooth muscle cells [115] Sphingosine 1-phosphate is also a very potent calcium mobilizing agonist in viable Swiss 3T3 fibroblast cells [116] To study further the biological activity of this potential second messenger, Kratzer and Schmidt achieved its synthesis [117] Specifically, the protected sphingosine derivative **194** was phosphitylated with bis-(2-cyanoethoxy)-*N,N*-diisopropylamino

phosphine (**77b**) and afforded, after aqueous iodine oxidation, the sphingosine phosphotriester **195** in 94% yield Treatment of **195** with *N,N*-dimethylamine in ethanol followed by tetra-*n*-butylammonium fluoride gave the desired sphingosine 1-phosphate **196** in *ca* 75% yield [117]



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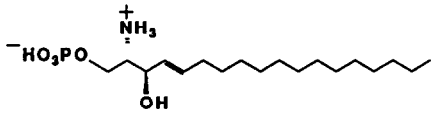

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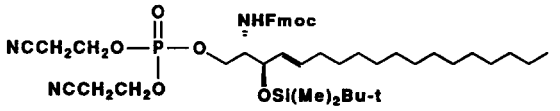
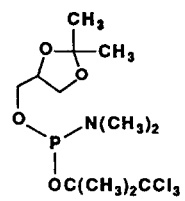


Fmoc= 9-fluorenylmethoxycarbonyl **195**

The synthesis of phosphatidylserines has been accomplished by condensation of the glycerophosphoramidite **197** with suitably protected serine derivatives in the presence of *N,N*-dimethylaniline hydrochloride [118a] After oxidation, the purified phosphotriester was treated with 70% perchloric acid and, subsequently, with tetradecanoyl chloride to give the acylated glycerophosphotriester **198** Treatment of **198** with lithium[Co(I) phthalocyanine] in methanol produced the glycerophosphatidyl-L-serine **199** in 30% yield.[118a] Similarly, the phosphitylation of a properly protected serine with chloro-(*N*-morpholino)methoxyphosphine led to a phosphoramidite derivative, which enabled the multistep synthesis of a lysophosphatidylserine with a digoxin-like 19 4 acyl group [118b]



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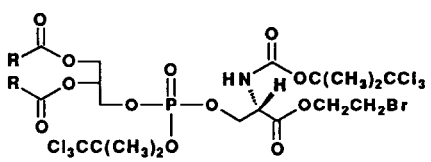
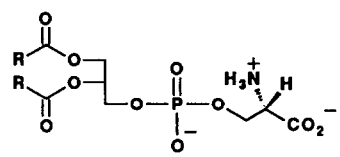
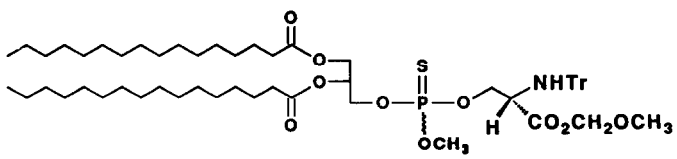


198 R= $C_{13}H_{27}$



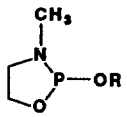
199 R= $C_{13}H_{27}$

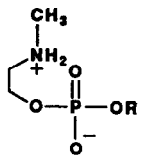
A phosphorothioate analogue of phosphatidylserine has been synthesized from the reaction of the glycerophosphoramidite **156** with *N*-trityl-L-serine methoxymethyl ester and 1*H*-tetrazole [107b,118c] Subsequent sulfurization with elemental sulfur in toluene generated the 1,2-dipalmitoyl-*sn*-glycero-3-thiophospho-L-serine derivative **200** The configuration of the deprotected phosphorothioate at phosphorus was assigned with respect to the stereospecific hydrolysis of the *Rp* isomer by phospholipase A_2 of bee venom [107b,118c]

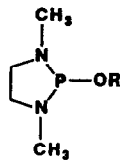


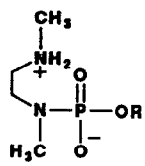
Tr= triphenylmethyl **200**

McGuigan *et al* have also investigated the synthesis of phospholipid analogues *via* phosphoramidite derivatives [119,120] The synthetic approach involved the preparation of cyclic

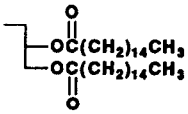






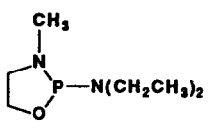


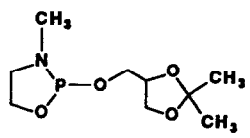
201a R= $C_{12}H_{25}$
b = $C_{18}H_{37}$
c = Δ^9-$C_{18}H_{35}$
d =

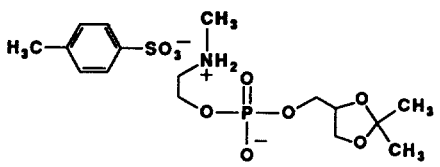


202a-d **203b-d** **204b-d**

phosphoramidites (**201a-d**) from 2-chloro-3-methyl-1,3,2-oxaphospholidine and the appropriate alcohols [119a-c] Oxidation of **201a-d** with dinitrogen tetroxide resulted in the corresponding cyclic phosphoramidate derivatives, which underwent facile hydrolytic cleavage to phosphate diesters (**202a-d**) [119a-c] Stumpf and Lemmen have similarly applied oxazaphospholanes to the synthesis of phospholipids [121] It must however be noted that these synthetic methods originated from the findings of Kodaira and Mukaiyama[122] published in 1966 These researchers demonstrated that the reaction of 1,2-acetoneglycerine with the phosphoramidite **205** gave the oxaphospholidine **206** in 74% yield Oxidation of **206** with dinitrogen tetroxide, and treatment with *p*-toluenesulfonic acid monohydrate afforded the glycerophosphatidyl ethanolamine **207** in yields exceeding 90% [122]

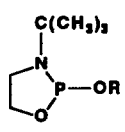


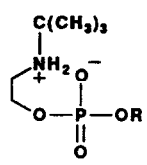




205 **206** **207**

The cyclic phosphoramidite derivatives **208a-c**, derived from 2-chloro-3-*tert*-butyl-1,3,2-oxaphospholidine, have alternatively produced *N*-substituted phospholipids (**209a-c**) upon hydrolysis of the corresponding cyclic phosphoramidate intermediates [119d] Additionally, the cyclic phosphorodiamidites **203b-d** have been prepared from 2-chloro-1,3-dimethyl-1,3,2-diazaphospholidine and led to the synthesis of ethylenediamine-derived phospholipids (**204b-d**) [119c,120] Mildly acidic conditions were required to induce ring opening of the parent cyclic phosphorodiamidate derivatives [119c,120] Furthermore, the oxidation of the phosphoramidites **208a-c** with dinitrogen

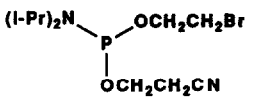




208a R= $C_{12}H_{25}$
b = $C_{18}H_{37}$
c = Δ^9-$C_{18}H_{35}$

209a-c

tetroxide and treatment of the resulting phosphoramidates with refluxing aqueous tetrahydrofuran effected both heterocycle cleavage and *N*-deprotection simultaneously The phosphatidyl ethanolamines **209a-c** were isolated in 72-92% yields [123] Incidentally, some nucleoside cyclic phosphoramidites and phosphoramidates have been synthesized, as potential inhibitors of thymidylate synthetase, from either 2-chloro-3-methyl-1,3,2-oxaphospholidine or 2-chloro-1,3-dimethyl-1,3,2-diazaphospholidine [124]



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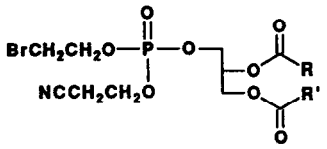
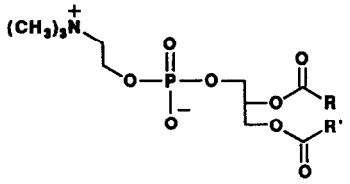
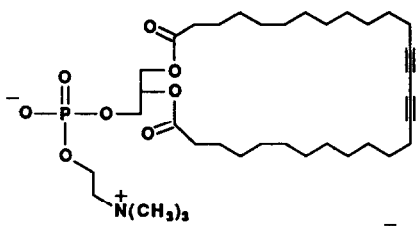




211 R= $C_{15}H_{31}$
R'= $CH_2(CH_2)_2CH(CH_2)_{12}CH_3$
OSi(Me2)Bu-t

212

A different approach to the preparation of phospholipids has been proposed by Hébert and Just [125a] Essentially, the reaction of the phosphoramidite **210** with 1,2-diacylglycerols afforded the glycerophosphotriester **211** after oxidation. Treatment of the phosphotriester with trimethylamine generated the zwitterionic phosphatidylcholine **212** in 92% yield While simplifying the purification of reaction intermediates, this approach did not promote the isomerization of diacylglycerols [125a] In a similar manner, the phosphoramidite **210** has been used in the synthesis of unusual macrocyclic and bolaform phosphatidylcholines (**213** and **214**, respectively) [125b]



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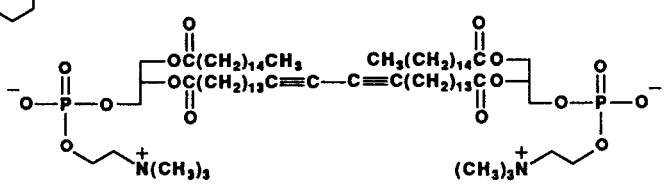
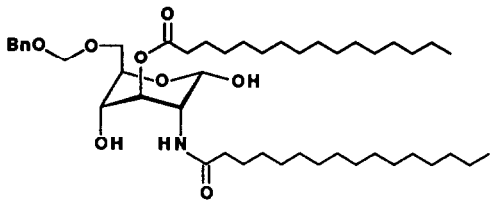


214

Benzyloxy-(2-cyanoethoxy)-*N,N*-diethylaminophosphine (**78**) has been especially useful in the phosphitylation of the *N*-acylated-D-glucosamine **215** [126a] Oxidation of the resulting diphosphite triester followed by removal of the benzyl protecting groups generated the 3-*O*-palmitoyl-2-deoxy-2-palmitamido-α-D-glucopyranose-1,4-diphosphate **216**, a Lipid A monosaccharide analogue [126a] Lipid A is a constituent of lipopolysaccharides of Gram-negative bacterial cell wall that has been shown to be a major causative agent in the induction of septic shock [126b]

The phosphitylation of a *N*-acylated-D-glucosamine derivative with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) in the synthesis of a Lipid X analogue has also been described by Balreddy *et al* [126c] Investigations regarding Lipid X and Lipid A analogues indicated that the number and position of the fatty acyl groups of Lipid A played an important role in the induction of septic shock [126d] Notably, Lipid X is a non-toxic biosynthetic intermediate of Lipid A.[126c]



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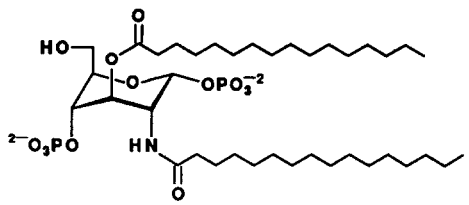
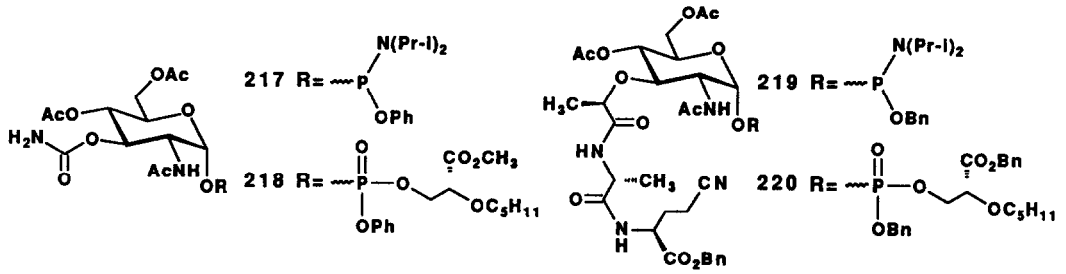


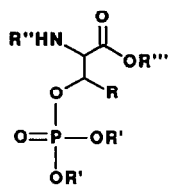
216

In an effort to design inhibitors of bacterial cell wall transglycosylation, a critical step in the construction of the polyglycan chains of peptidoglycans, Hecker *et al* [127] synthesized the glucopyranosyl phosphoramidites **217** and **219**, which upon condensation with the proper glycerate-pentyl ether afforded the sugar-phosphate-glycerate ethers **218** and **220** after oxidation Unfortunately, the deprotected and purified derivatives did not exhibit antibacterial activity [127]

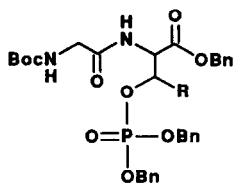


2 5 Phosphopeptides and Glycophosphopeptides

An important application of phosphoramidite derivatives relates to protein phosphorylation There is increasing evidence that protein phosphorylation is a regulatory element in carcinogenesis mediated by protein kinases [128] The synthesis of phosphorylated peptides may therefore provide insight on the mechanism by which phosphorylation affects the structure of peptides and proteins A model reaction for the phosphorylation of hydroxyamino acids has been proposed by Perich and Johns [129a,b] The reaction consisted of the phosphitylation of simple alcohols (methanol, isopropyl alcohol and *tert*-butyl alcohol) with bis-(benzyloxy)-*N,N*-diethylaminophosphine (**76c**) or bis-(*tert*-butyloxy)-*N,N*-diethylamino phosphine (**76e**) in the presence of 1*H*-tetrazole Oxidation of the resulting trialkyl phosphites with MCPBA produced the corresponding phosphate esters in high isolated yields (97-99%) [129a] Bis(*tert*-butyloxy)-*N,N*-diethylaminophosphine (**76e**) has also proven useful in the phosphitylation of phytanol and lauryl alcohol toward the synthesis of novel acceptor substrates for a mannosyl transferase [130] These model reactions led to the phosphorylation of serine[129c,131,132] and threonine [131] In fact, the phosphorylated amino acids **221-223** were obtained from the reaction of



221 R= H, R'= R'''= Bn, R''= Boc
222 = Me, = R'''= Bn; = Boc
223 = H, = tert-Butyl, = allyloxycarbonyl,
R'''= 4-nitrobenzyl

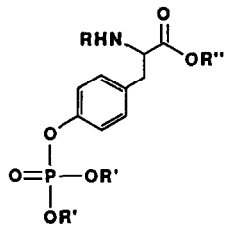


224 R= H
225 = Me

Boc= tert-butoxycarbonyl

suitably protected serines or threonines with **76c**[131] or **76e**[132] followed by oxidation Upon cleavage of the Boc groups and addition of the hydroxybenzotriazole ester of Boc-Ala at pH 7-8, the phosphorylated peptides **224** and **225** were isolated in yields exceeding 80% [131]

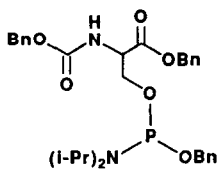
The phosphorylation of tyrosine derivatives with similar bis-(alkoxy)-*N,N*-diethylaminophosphines has also been effective For example, the *O*-phosphotyrosine derivatives **226-230** were isolated in high yields from protected tyrosine precursors [129c,d,133a-c] The phosphotyrosines **226a**, **227** and **228** were applied to the synthesis of various phosphotyrosine-containing peptides [129c,d,133a,c]



226a R= Boc; R'= benzyl, R"= 4-nitrobenzyl
b = Boc, = benzyl; = H
227 = Fmoc, = tert-butyl, = H
228 = Fmoc, = methyl, = Maq
229 = Boc; = methyl; = H
230 = Boc; = ethyl; = H

Maq= 2-(9,10-dioxo)anthrylmethyl

In an alternate approach, the phosphitylation of a partially protected L-serine derivative with bis-(*N,N*-diisopropylamino)benzyloxyphosphine (**79b**) resulted in the formation of the phosphoramidite derivative **231** The coupling of **231** with a L-threonine derivative, and oxidation of the resulting phosphite triester with *tert*-butyl hydroperoxide, gave the serylthreonyl phosphate triester **232** in 80% yield [134]



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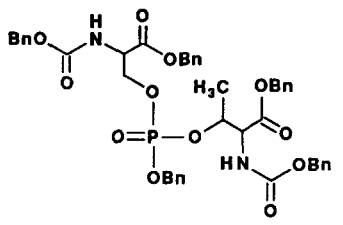
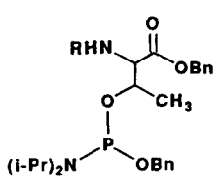
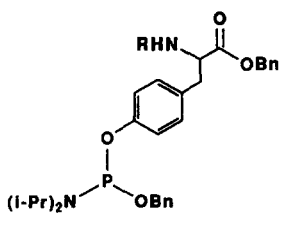


232

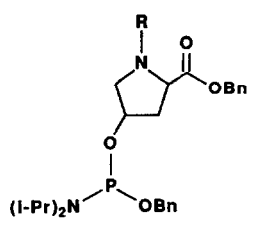
The hydroxyamino acid phosphoramidites **233-235** were similarly prepared from the *N*-benzyloxycarbonyl derivative of threonine, tyrosine, and hydroxyproline benzyl esters, respectively, and **79b** [135] These phosphoramidites were employed in the synthesis of various phosphate diesters Thus, the reaction of **231** with 3'-*O*-acetylthymidine or the condensation of **233** with N_{α}-(benzyloxycarbonyl) valylserylisoleucyl $C_{\alpha-1}$-benzyl ester afforded, after oxidation, the phosphotriester **236** or **237** in 95% or 87% yield, respectively [135]



233 R= CO_2Bn

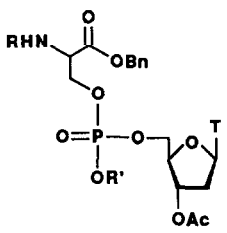


234 R= CO_2Bn

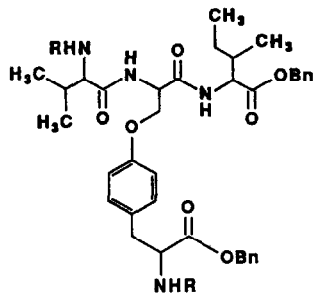


235 R= CO_2Bn

Evidence supporting the occurrence of a phosphodiester function that links the hydroxyl groups of serine and threonine residues in *Azotobacter flavodoxin* proteins has recently been corroborated by

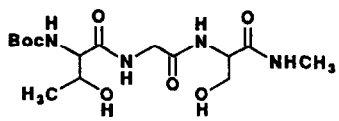


236 R= CO_2Bn



237 R= CO_2Bn

NMR spectroscopy [136] Consequently, **232** can serve as a model to study the spectroscopic and chemical features of an intermolecular phosphodiester linkage The possibility of generating an intramolecular phosphodiester link between a serine and a threonine has been examined by van Oijen *et al* [136] The partially protected peptide Thr-Gly-Ser **238** was treated with 4-chlorobenzyloxy-bis-(*N,N*-diisopropylamino)phosphine and 1*H*-tetrazole Oxidation of the reaction mixture with *tert*-butyl hydroperoxide produced the cyclic phosphopeptide **239** Intramolecular phosphodiester linkages could affect the structure of these molecules and may lead to the generation of molecular hosts having interesting structural features and binding properties



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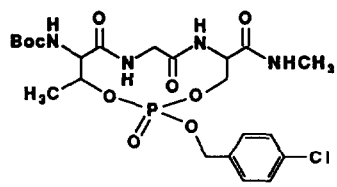


239

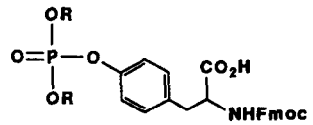
de Bont *et al* [137a] described the automated solid-phase synthesis of a pentapeptide (H-Lys-Arg-Thr-Leu-Arg-OH) containing the phosphorylation site of the epidermal growth factor receptor The threonine residue was incorporated into the peptidic chain without hydroxyl protection Treatment of the solid-phase bound peptide with bis-(4-chlorobenzyloxy)-*N,N*-diisopropylaminophosphine (**77d**) produced, after oxidation, deprotection and purification, the phosphopeptide H-Lys-Arg-Thr-$(PO_3)^{-2}$-Leu-Arg-OH which was identical to that prepared in solution phase [137a,b] This methodology has further been applied to the solid-phase synthesis of *O*-phosphoserine and *O*-phosphothreonine-containing peptides along with their phosphorothioate analogues [137d]

Bannwarth and Trzeciak[138a] have independently shown that the hydroxyl group of the serine residue in the pentapeptide Boc-Asp(OBn)-Ala-Ser-Gly-Glu$(OBn)_2$ was easily phosphitylated with bis-(benzyloxy)-*N,N*-diisopropylaminophosphine (**77a**) Similarly, bis-(allyloxy)-*N,N*-diisopropylamino phosphine (**77c**) effected the *O*-phosphitylation of properly protected serine, tyrosine and threonine derivatives in addition to the serine residue of the peptide Z-Asp(OBu-t)-Ala-Ser-Gly-Glu$(OBu\text{-}t)_2$ [138b] The amidite **77a** has additionally been employed for the *O*-phosphitylation of peptides anchored to a solid support Following oxidation with *tert*-butyl hydroperoxide, the deprotection of amino acid side chains and the release of peptides from the stationary phase were accomplished by specific trifluoroacetic acid formulations [139a] This method led to the synthesis of various *O*-phosphopeptides (up to 15 residues in length) in high yields

Dibenzyl-*N,N*-diethylphosphoramidite (**76c**) has alternatively been used in the phosphitylation of the hydroxy function of either protected serine derivatives or multiple serine-containing peptides [140]

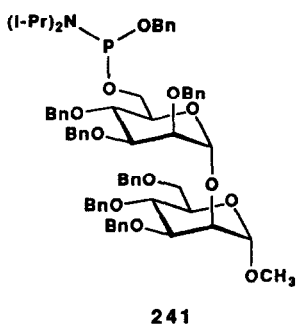
However, because of the sensitivity of the benzyl phosphate protecting group to the acidic conditions used in peptide synthesis,[129d] the utilization of **77d** in the synthesis of phosphopeptides has been suggested [137a,c] Perich *et al* [141] and Lacombe *et al* [132] subsequently reported that besides dibenzyl-*N,N*-diethylphosphoramidite (**76c**),[141a-c] diphenyl- (**76f**),[141a,b] dimethyl- (**76a**),[141a,b] diethyl- (**76b**),[141a,b,i] di-(4-bromobenzyl)- (**76g**),[141d] and di-*tert*-butyl- (**76e**)[132,141a,b] *N,N*-diethylphosphoramidites can be used for the efficient phosphitylation of the hydroxyl group of protected serines Given the stability of the phenyl phosphate function during peptide synthesis and the facile removal of the phenyl group by hydrogenolysis, Boc-Ser(PO_3Ph_2)-OH and Boc-Thr(PO_3Ph_2)-OH have been recommended for the synthesis of peptides containing *O*-phosphoserine [141a,e,f,h] and *O*-phosphothreonine residues [141g]

Kitas *et al* [142a,b] reported the phosphitylation of tyrosine derivatives with bis-(methoxy)-*N,N*-diethylaminophosphine (**76a**) Following oxidation with MCPBA, the incorporation of Fmoc-Tyr(PO_3Me_2)-OH or Boc-Tyr(PO_3Me_2)-OH synthons into peptides has been described Several deprotection procedures performed in the presence of thioanisole were found effective in the demethylation of Tyr(PO_3Me_2)-containing peptides [142b] The phosphitylation of resin-bound tyrosine-containing peptides with the phosphoramidites **76a**,[142c] **77a**,[139a,142c,143] **77c**,[142c] **77e**,[142c,143] and **77f**[139b] or bis-(*tert*-butyloxy)-*N,N*-diethylaminophosphine (**76e**)[144,145] has also been achieved This "global" phosphorylation approach yielded results comparable to those obtained from the incorporation of O^4-phospho-L-tyrosine building blocks (**240a-d**) during solid-phase peptide synthesis [142c,d,143] It has additionally been reported that **76e** led to the efficient global phosphorylation of peptides containing multiple serine and/or tyrosine/threonine residues [145,146]



240a R= methyl
b = benzyl
c = allyl
d = tert-butyl

The phosphitylating reagent bis-(*N,N*-diisopropylamino)benzyloxyphosphine (**79b**) has been useful in the preparation of the dimannosyl phosphoramidite **241** toward the synthesis of the peptidyldimannosyl phosphate **242** [147] The glycophosphopeptide **242** contains the conserved carboxy-terminal Lys-Asp of the glycosylated phosphatidylinositol-anchor of *Trypanosoma brucei* variant-specific surface glycoprotein [147]



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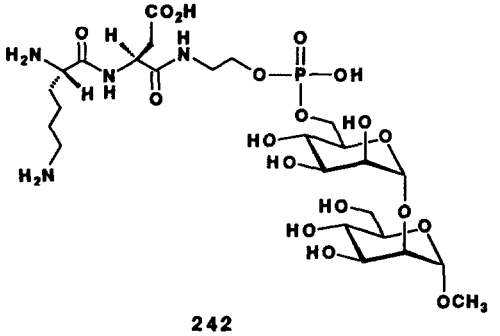
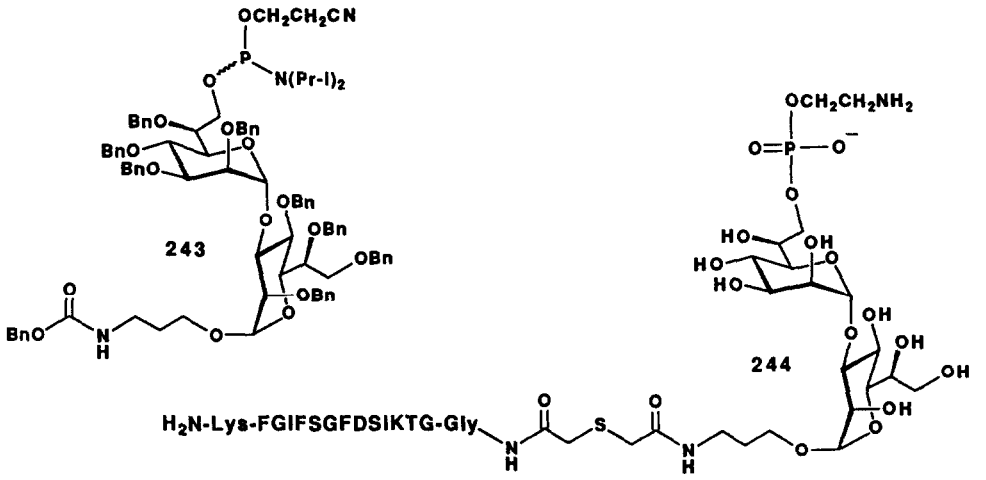


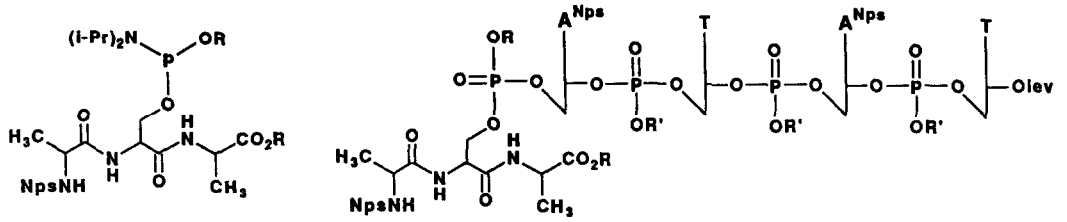
242

The bis-amidite **79b** has also been used in the preparation of the disaccharide phosphoramidite **243**, which led to the synthesis of the sugar-peptide conjugate **244** [148] This conjugate may generate valuable immunological properties toward the development of a synthetic vaccine against *Neisseria Meningitidis*



2 6 Nucleopeptides and Oligonucleotide-Peptide Conjugates

The facile access to phosphitylated peptides in the synthesis of nucleopeptides was demonstrated by Kuyl-Yeheskiely *et al* [149] Specifically, the reaction of bis-(*N,N*-diisopropylamino)allyloxyphosphine (**79e**) with the tripeptide NPS-Ala-Ser-Ala-OAllyl generated the peptidyl phosphoramidite **245** Condensing **245** with the 5'-hydroxy function of a *N*-protected tetranucleoside 2-chlorophenyl phosphotriester afforded, after oxidation, the protected nucleopeptide **246** in 90% yield

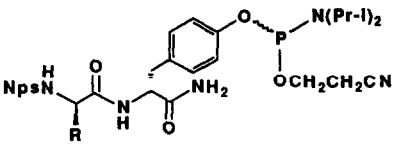


245 R= allyl

Nps= (2-nitrophenyl)sulfenyl

246 R= allyl,
R'= 2-chlorophenyl

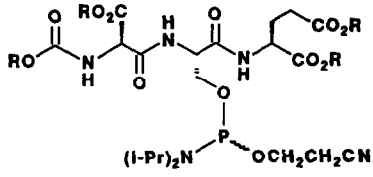
In recent reports, the solid-phase synthesis of similar nucleopeptides has been described [150,151] N^{α}-(2-Nitrophenylsulfenyl)phenylalanyl tyrosine amide[150] and N^{α}-(2-nitrophenylsulfenyl)alanyl tyrosine amide[151] were converted to the respective phosphoramidites **247** and **248** upon treatment with bis-(*N,N*-diisopropylamino)2-cyanoethoxyphosphine (**79d**) and 1*H*-tetrazole The phosphoramidite **247** was then combined with the 5'-terminus of a tetradeoxyribonucleotide covalently linked to a CPG support This strategy eventually led to H-Phe-Tyr(pATAT)-NH_2, a fragment of the nucleoprotein formed in the early stage of the bacteriophage øX174 rolling circle replication of double-stranded circular DNA.[150]



247 R= CH_2Ph
248 = CH_3

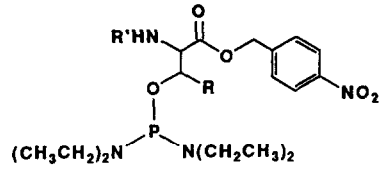
The activated phosphoramidite **248** has alternatively been treated with the 5'-OH function of a protected heptaribonucleotide anchored to a solid support After deprotection and purification, the RNA-nucleopeptide H-Ala-Tyr(pUUAAAAC)-NH_2 corresponding to a VPg nucleoprotein fragment of the poliovirus was isolated [151]

The naturally occurring DNA-nucleopeptide H-Asp-Ser[5'-pAAAGTAAGCC-3']-Glu-OH (*Bacillus subtilis* phage ø29) has been synthesized by incorporation of the phosphoramidite **249** at the 5'-end of a solid-phase bound decadeoxyribonucleotide [152] Due to the sensitivity of the serine-phosphate function to bases, the 2-(*tert*-butyldiphenylsilyloxymethyl)benzoyl group was used to protect the exocyclic amino group of the DNA nucleobases, while the oligomer was anchored to the solid support *via* a base-labile oxalyl linker [152] Thus, treatment of the support with 0 25 M tetra-*n*-butylammonium fluoride in pyridine-water effected the release of the DNA-nucleopeptide from the solid phase and the removal of cyanoethyl and nucleobase protecting groups The deblocking of peptidic 4-nitrobenzyl and 4-nitrobenzyloxycarbonyl protecting groups was also accomplished, under mild conditions, with sodium dithionite and sodium bicarbonate [152]

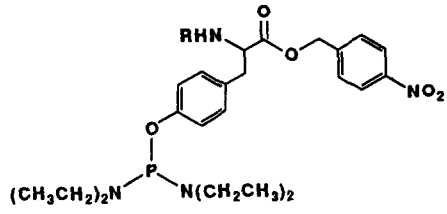


249 R= 4-nitrobenzyl

Of interest, the phosphorodiamidite derivatives of serine, threonine and tyrosine (**250a-b, 251**) were efficiently converted to their corresponding H-phosphonates upon acidolysis These derivatives were also applied to the synthesis of nucleopeptides [153]

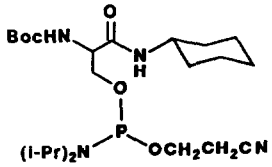


250a R= H, R'= CO_2Bn
b = Me, = CO_2Bn



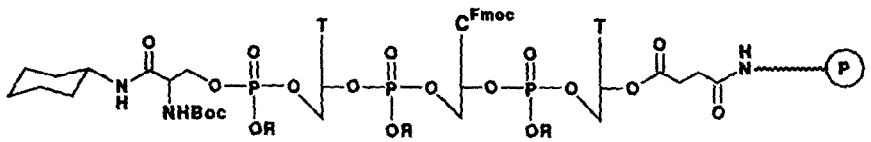
251 R= CO_2Bn

The synthesis of a model nucleopeptide having a phosphodiester function joining the 5'-terminus of trinucleotide to a serine residue has been reported by Robles *et al.*[154] The serine-derived phosphoramidite **252** was activated with 1*H*-tetrazole and treated with the 5'-hydroxy function of a trinucleotide assembled on a polystyrene support After aqueous iodine oxidation, the nucleopeptide



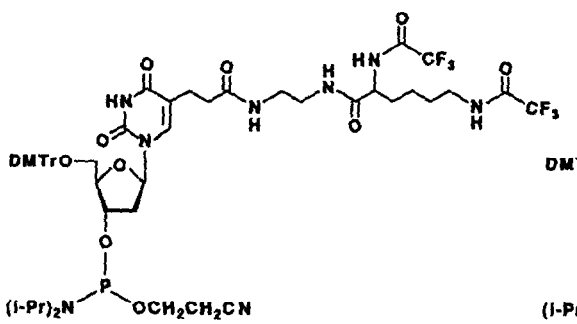
252

253 was partially deprotected and released from the support by treatment with 0.05 M potassium carbonate in methanol·dioxane (1 1) at 20 °C [154] This approach is thus recommended for the synthesis of nucleopeptides with base-labile phosphodiester functions

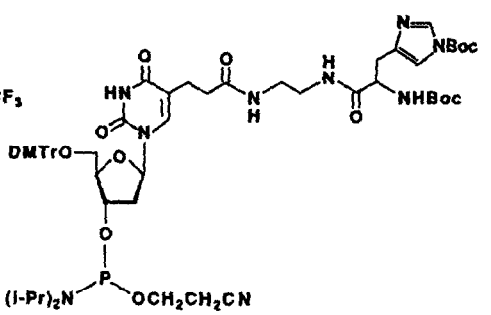


253 (P) = polystyrene; R= CH_2CH_2CN

The incorporation of amino acid or peptide residues into oligonucleotides has also been accomplished through the phosphoramidites **254** and **255** by standard solid-phase synthesis [155] While the synthesis of L-lysine-DNA conjugates may provide valuable information regarding the transport of DNA into cells, the conjugation of imidazole to DNA segments may catalyse the sequence-specific hydrolysis of RNA. The outcome of these potential applications remains to be known

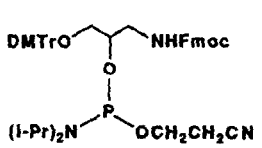


254

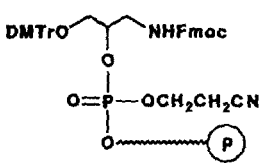


255

The facile preparation of 3'-oligonucleotide-peptide conjugates has been described [156] The synthetic approach consisted of the reaction of a commercial teflon resin with the phosphoramidite linker **256** activated with 1*H*-tetrazole. After oxidation, the Fmoc group was removed from the support **257** and the stepwise synthesis of either Z-D-Phe-L-Phe-Gly, $(Lys)_5$ or $(Trp)_5$ was undertaken Upon completion of the final peptidic addition, the 4,4'-dimethoxytrityl group was cleaved from **257** under acidic conditions, and solid-phase oligonucleotide synthesis was initiated by coupling deoxyribonucleoside phosphoramidite monomers Due to the lability of peptides to concentrated ammonium hydroxide, oligonucleotidic deprotection was effected with ethylenediamine in absolute ethanol (1 1) for 1 h at 55 °C. Interestingly, the Boc groups of the DNA-lysine conjugate were cleaved by treatment with 90% trifluoroacetic acid/ethanedithiol for 5 min without significant depurination [156]



256



257

(P) = teflon support

These methodologies demonstrated the suitability and practicability of phosphoramidite intermediates in the preparation of biologically important nucleopeptides

CONCLUDING REMARKS

The application of phosphoramidite derivatives to the phosphorylation of non-nucleosidic biomolecules has been emphasized in this Report and has further demonstrated the efficiency and versatility of phosphoramidite synthons In spite of the colossal influence phosphoramidite derivatives have had on the synthesis of oligonucleotides and their analogues[1-3] to benefit biomedical research, the phosphoramidite approach still requires further improvements For example, ribonucleoside phosphoramidite monomers, presumably because of stereoelectronic and steric factors, are not as efficient as the corresponding deoxyribonucleoside phosphoramidites in solid-phase oligonucleotide synthesis This limitation also applies to a number of modified nucleoside phosphoramidites[3] Thus, improving the chemical reactivity of ribonucleoside phosphoramidites may not only provide easier access to branched or catalytic RNA molecules but may as well facilitate the synthesis of specific oligonucleotide analogues

Should the application of natural and/or modified oligonucleotides as therapeutics agents become a reality, the economics of large-scale oligonucleotide synthesis will become important and will undoubtedly rely on the efficiency of synthetic methods.

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