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Phosphonate analogues of nucleoside polyphosphates

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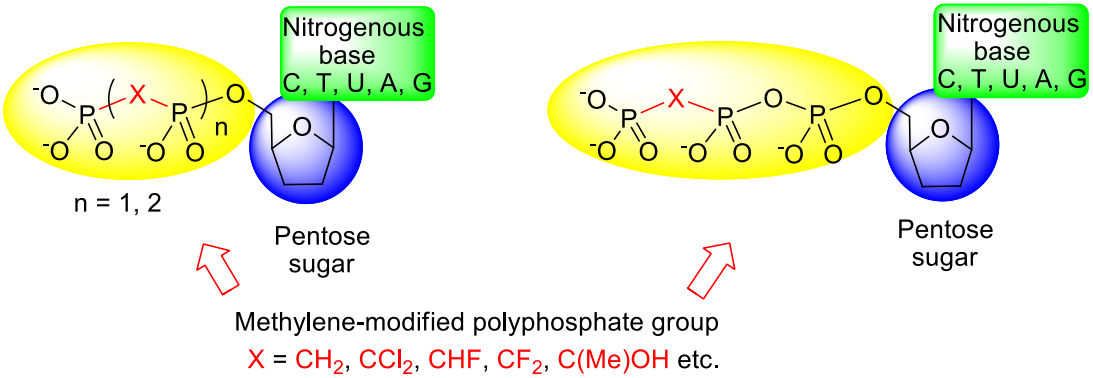
Abstract

This article provides an overview of the efforts toward the synthesis of nucleoside polyphosphate mimics featuring a P-CXY-P scaffold. The following synthetic approaches to these compounds are summarized: (i) nucleophilic displacement of 5´-*O*-tosyl nucleoside by the ammonium salts of methylenebisphosphonic acid; (ii) synthesis via activated phosphate/phosphonate substrates; (iii) Mitsunobu coupling between a nucleoside and methylenebisphosphonic acid; (iv) phosphorylation of a protected nucleoside under Yoshikawa's reaction conditions with methylenebis(phosphonic dichloride); (v) synthesis via nucleophilic cleavage of cyclic trimetaphosph(on)ates; (vi) enzyme-mediated reactions.



Keywords: Pyrophosphate analogues, modified nucleotides, bisphosphonates, multicomponent reactions, phosphonate carbanions

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1. Introduction

The intrinsic stability of methylene-modified polyphosphates toward both enzymatic and chemical hydrolysis makes them a useful tool for investigation of biological processes involving phosphate substrates. Nucleotide analogues featuring methylenebisphosphonate linkage are especially interesting since they represent a versatile platform for the synthesis of enzyme inhibitors and important agents for anticancer and antiviral therapy.[1-5] Progress in the development of these species is closely linked with the development of a bioisostere concept whereby methylenebisphosphonates (H_2O_3P-CXY-PO_3H_2) are analogues of endogenous pyrophosphate (H_2O_3P-O-PO_3H_2).[6,7] During recent decades numerous fundamental researches were made on design and tactical application of phosphate and pyrophosphate isosteres.[8-20] The present review focuses on synthetic repertoires available for the construction of nucleoside polyphosphate analogues incorporating P-CXY-P scaffold. It is a continuation of previous communications from the authors' group that summarized advances in the synthesis of phosphonate-based bioisosteres.[21-24]

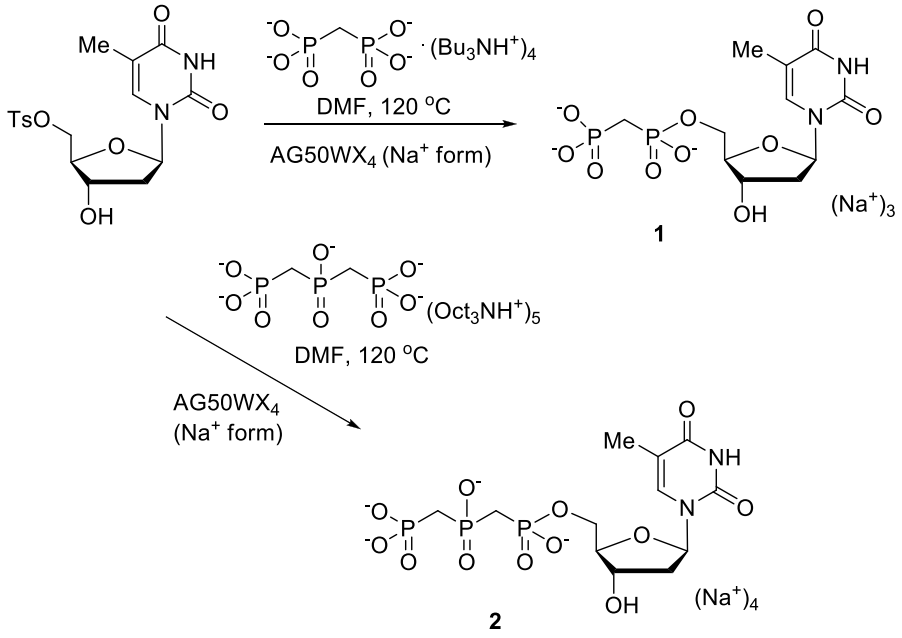
2. Preparation of Nucleoside Polyphosphate Analogues Featuring a P-CXY-P Scaffold

2.1. Synthesis via 5´-*O*-tosyl nucleosides

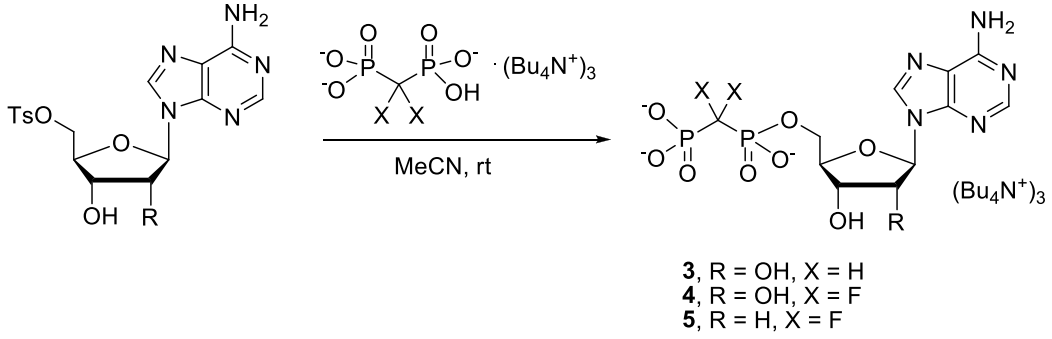
Lipophilic salts of methylenebisphosphonic acid such as tris(tetra-*n*-butylammonium) bisphosphonate are good reagents for synthesis of nucleotide analogues from nucleosides by direct nucleophilic displacement at the 5´-position of 5´-*O*-tosyl nucleosides. While this is multi-step synthesis (particularly if protected nucleosides are used), the relative simplicity and high reliability of the procedure make it a good supplement to existing methods based on addition of the nucleophilic 5'-hydroxyl group to activated phosphonate derivatives.

The preparation of methylene-modified nucleoside polyphosphates by the nucleophilic displacement of *O*-sulfonyl groups such as *p*-toluenesulfonyl (Ts) or methylsulfonyl (Ms), was first recorded by Stock in 1979 when it was reported that the action of trialkylammonium salts of methylenebisphosphonic acids on 5´-*O*-tosyl thymidine in DMF leads to the corresponding bisphosphonate analogues of thymidine di- and triphosphate **1**,

2 (Scheme 1).[25] Later, this approach has been adapted by Poulter and co-workers to the preparation of a variety of nucleoside diphosphates and their bisphosphonate analogues.[26] Thus, reactions between 5´-*O*-tosyl derivatives of adenosine and 2´-deoxyadenosine and tris(tetra-*n*-butylammonium)salts of methylenebis-phosphonic acids in freshly distilled dry acetonitrile afforded the nucleoside bisphosphonates **3**-**5** in good yields (Scheme 2).[27] By essentially the same procedure (sometimes including variations in the reaction conditions), CHF- and CF_2-analogues of 2´-deoxythymidine diphosphate[28], CF_2-modified guanosine diphosphate[29] and adenosine 5'-(α,β:β,γ-dimethylenetriphosphate)[30] have been synthesized and characterized by spectral methods. Findings on the selectivity of the series of modified ATPs for rat $P2X_2$ and $P2X_{2/3}$ receptors are summarized in ref. 15.



Scheme 1. Synthesis of bisphosphonate analogues of thymidine di- and triphosphate from 5´-*O*-tosyl-thymidine.

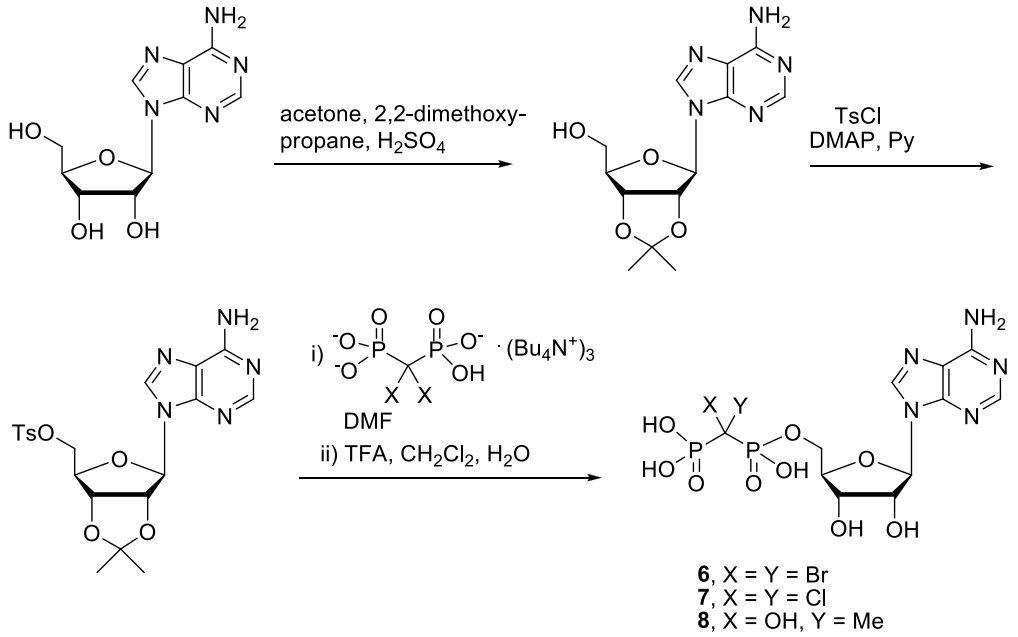


Scheme 2. Poulter's synthesis of α,β-methylene-modified ADP derivatives.

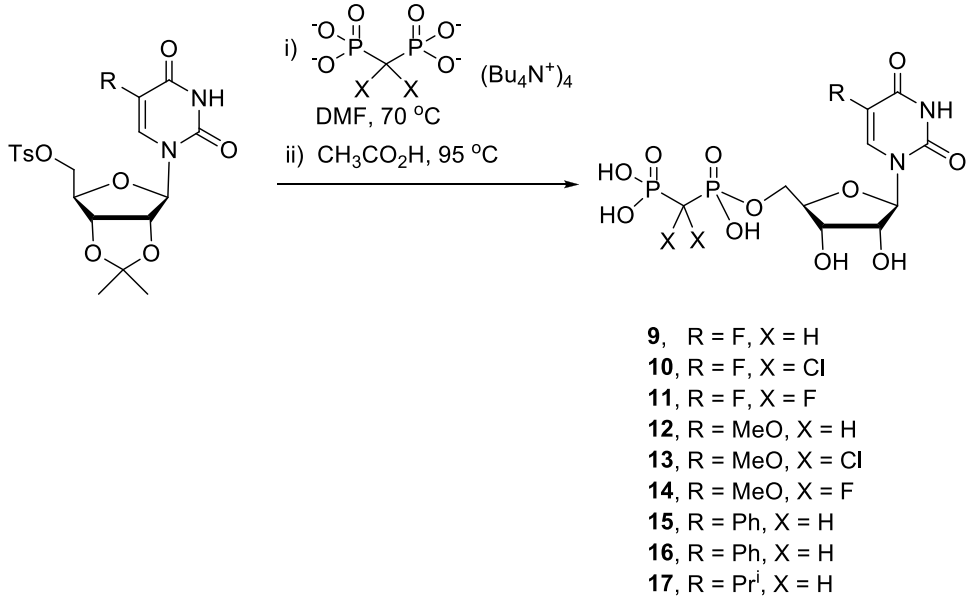
An attractive feature of Poulter's phosphorylation method is that either protected or unprotected nucleosides can be phosphorylated. Yields are higher for protected nucleosides, suggesting that in appropriate cases it may be worthwhile to use protected strategy in spite of the added synthetic steps.[31] Thus, α,β-methylene-modified derivatives of ADP **6**-**8** were prepared using 2´,3´-*O*-isopropylidene-adenosine 5´-*O*-

tosylate. After purification of the intermediates they were deprotected by treatment with 6-8% trifluoroacetic acid to obtain desired products (Scheme 3).[32]

With a view to set rules for design of UDP-based reversible $P2Y_6$ receptor antagonists as potential drugs, a variety of protected uridine 5´-tosylates were tested in nucleophilic displacement reactions with tetrabutylammonium bisphosphonates giving the desired uracil nucleotide analogues **9**-**17** after acidic work-up (Scheme 4).[33]

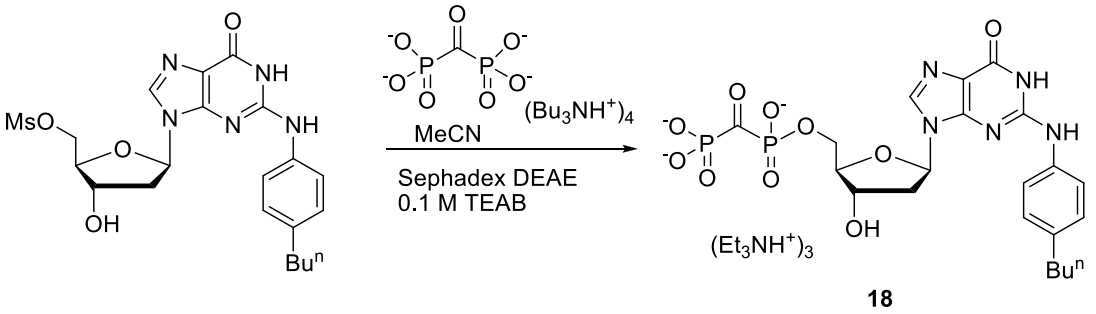


Scheme 3. Synthesis of α,β-methylene-substituted ADP derivatives via 2´,3´-*O*-isopropylidene-adenosine 5´-*O*-tosylate.



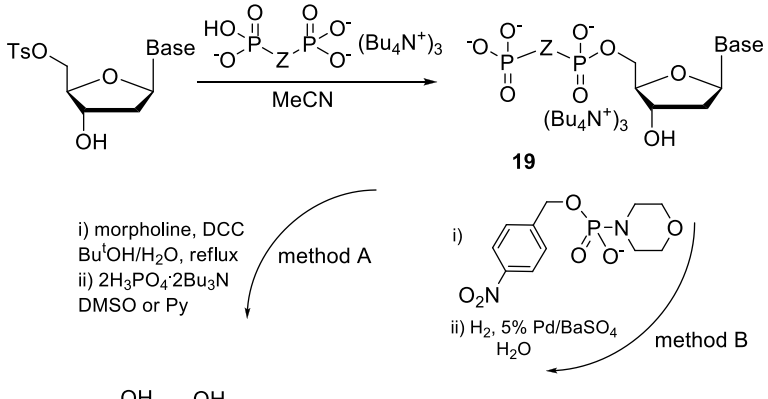
Scheme 4. Synthesis of uracil nucleotide analogues.

A new type of nucleoside polyphosphate analogues in which pyrophosphate oxygen is replaced by a potentially reactive carbonyl group was obtained by displacement of the 5′-mesyl group of the corresponding 5′-mesylnucleoside with carbonylbisphosphonate. Reaction of the tributylammonium salt of carbonylbis-phosphonate with N^2-(4-butylphenyl)-5′-mesyl-2′-deoxyguanosine in acetonitrile gave **18** isolated as a yellow solid in 93% by ion-exchange chromatography. This compound was a potent, competitive inhibitor of human DNA polymerase α (Scheme 5).[34]



Scheme 5. Synthesis carbonylbisphosphonate analogue of BuPdGDP via displacement of 5′-mesyl group.

Blackburn and Langston prepared α,β-substituted phosphonate analogues of 2′-deoxyadenosine and 2′-deoxythymidine 5′-triphosphates by a two-step reaction starting from the appropriate 5′-*O*-tosyl deoxy-nucleosides (Scheme 6).[28] In the first step, they prepared 2′-deoxynucleoside 5′-diphosphate analogues **19** in yield around 50-60%. The γ-phosphate group was attached subsequently either via activation of P^{β} of the dNDP as its morpholidate followed by reaction with inorganic phosphate (method A) or phosphorylation of nucleoside 5′-diphosphate with an excess of *p*-nitrobenzyl phosphoromorpholidate (method B). The *p*-nitro-benzyl group was removed by catalytic hydrogenolysis to give the dNTP analogues **20** in good yields.

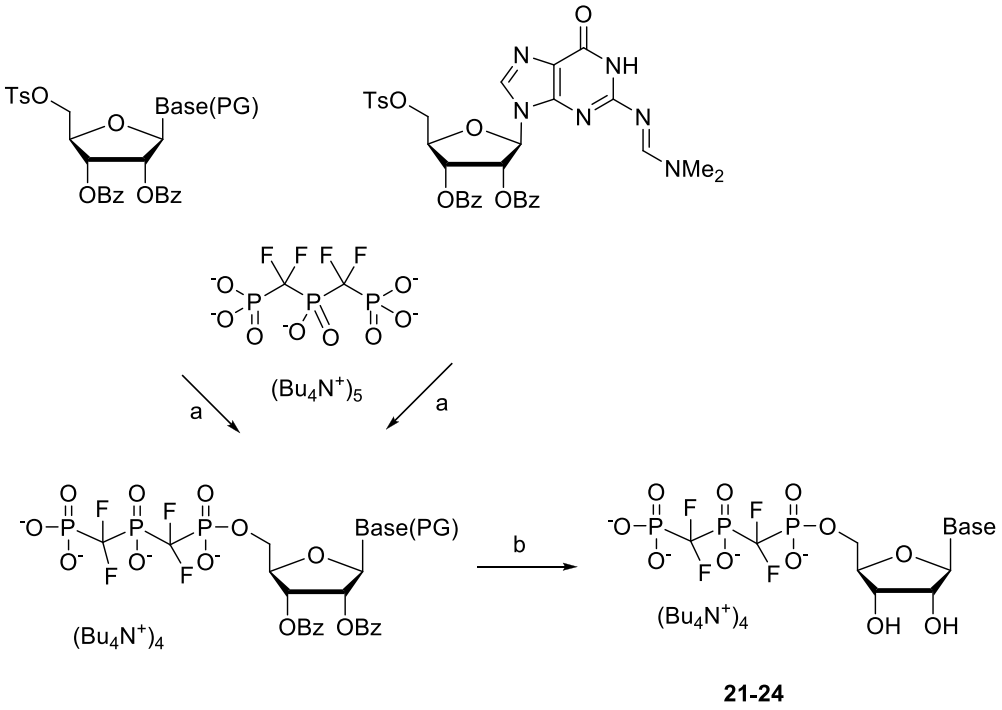


Base	Z	Yield (%)[a]
T	CH_2	32
T	CHF	28
T	CF_2	56
A	CH_2	58
A	CHF	53
A	CF_2	67

[a] Yields reported for *p*-nitrobenzyl phosphoromorpholidate

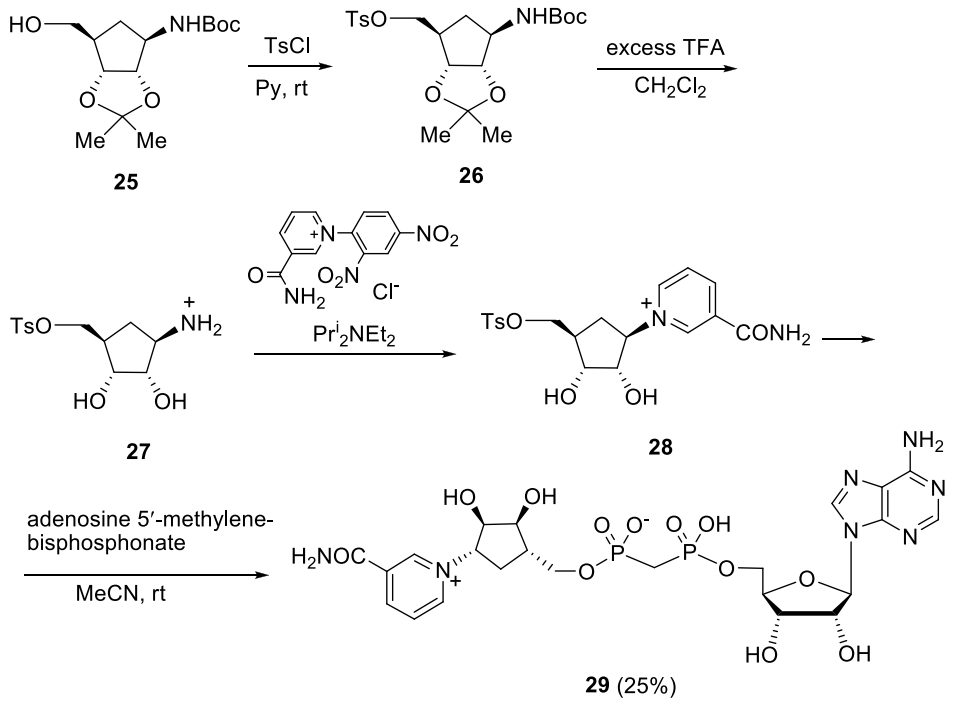
Scheme 6. Synthesis of α,β-CXY dNTP analogues by combination of tosylate substitution and phosphoromorpholidate protocol.

α,β:β,γ-BisCF_2 substituted RNA nucleotide analogues **21**-**24** potentially stable to enzymatic hydrolysis in RNA and DNA polymerase assay were prepared via nucleophilic displacement of 5´-tosylate in benzoyl protected nucleosides by the tetra-*n*-butylammonium salt of bis(difluoromethylene)triphosphonic acid (Scheme 7). Two equivalents of the tosyl nucleoside were required to ensure maximum consumption of triphosphonate salt. In the case of ATP, CTP and UTP nucleotide analogues authors were able to achieve conversion close to 90%, although in case of GTP analogue conversion did not exceed 20%. Preliminary biological results have shown that this class of nucleotides with modified triphosphate moiety revealed the correct polarity and minimal steric effects compared to the natural molecules.[35]

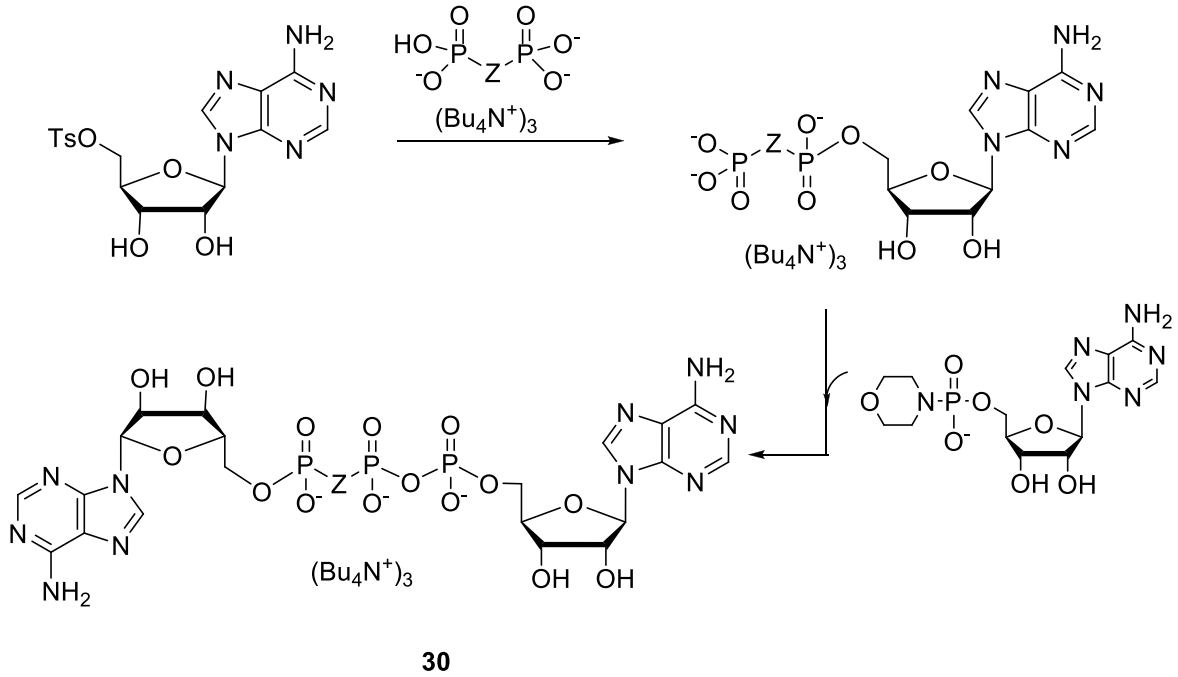


Scheme 7. Preparation of α,β:β,γ-bisCF_2-NTP analogues. Reactions conditions: (a) DMF, 110 °C; (b) NH_4OH, MeOH. Yields of bisCF_2-NTP analogues: α,β:β,γ-bisCF_2-ATP (**21**) 29%, α,β:β,γ-bisCF_2-CTP (**22**) 33%, α,β:β,γ-bisCF_2-UTP (**23**) 22%, α,β:β,γ-bisCF_2-GTP (**24**) 6%.

A stereospecific synthesis of methylenebisphosphonate carbocyclic analogue **29** of nicotinamide adenine dinucleotide (NAD^+) has been prepared in four steps (Scheme 8). Tosylation of **25** using *p*-toluenesulfonyl chloride in pyridine gives the key precursor **26** which was deprotected in one step by heating with excess TFA in dichloromethane. Reaction between **27** and *N*-(2,4-dinitrophenyl)-3´-carbamoylpyridinium chloride (Zincke reagent) in methanol afforded the pyridinium salt **28** which was further transformed by coupling with adenosine 5´-methylenebisphosphonate into carbocyclic analogue **29** of NAD^+.[36]



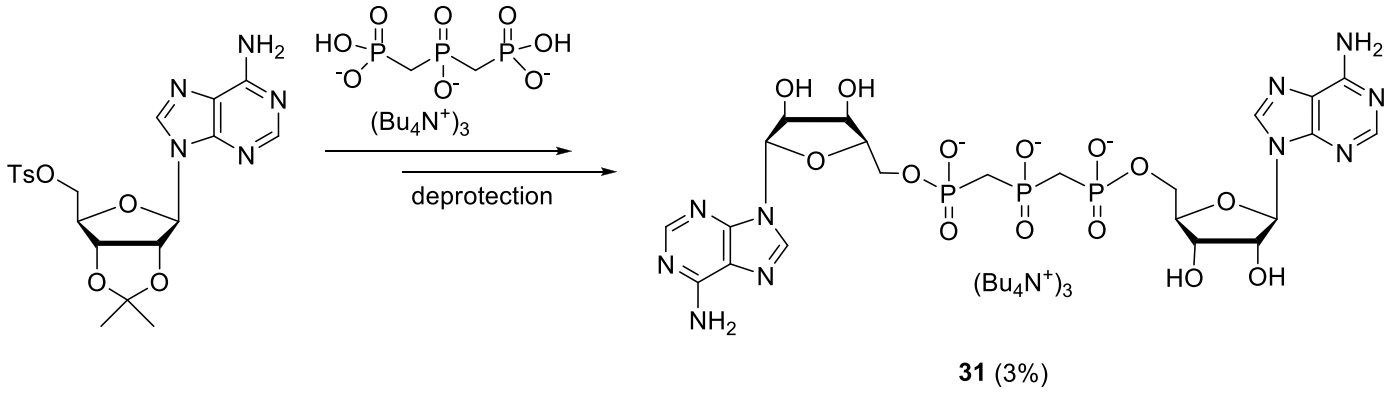
Scheme 8. Synthesis of methylenebisphosphonate analogue of NAD^+



Scheme 9. Synthesis of α,β-methylene-bridged analogues of Ap_3P.

As part of a program to investigate the mechanism of action of dinucleoside polyphosphate hydrolases, British biochemists described the synthesis of a range of analogues of diadenosine 5′,5′′′- triphosphate (Ap_3A).[37] The most effective route to compounds **30** involves the condensation of α,β-methylene analogues of

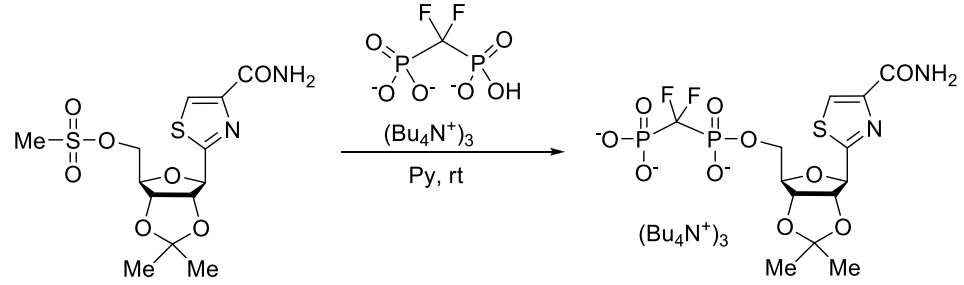
ADP, conveniently prepared by the method of Poulter, with adenosine 5′-phosphoromorpholidate (Scheme 9). The α,β:β,γ-bismethylene analogue **31** was prepared by the reaction between 2′,3′-*O*-isopropylidene adenosine 5′-tosylate and bis(dihydroxyphosphonomethyl)phosphinic acid. Unfortunately, the yield of pure material was only 3% (Scheme 10).[37]



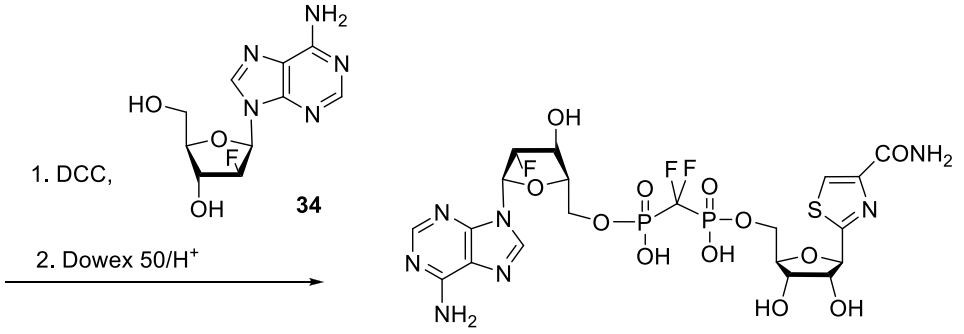
31 (3%)

Scheme 10. Synthesis of α,β:β,γ-bismethylene Ap_3A analogue via 5′-tosylate substitution.

Pankiewicz and co-workers have reported a one-pot reaction involving initial displacement of the mesyl group of 2′,3′-*O*-isopropylidene-5′-*O*-mesylthiazofurin (**32**) with the tris(tetrabutylammonium salt) of difluoromethylenebisphosphonic acid followed by DCC-coupling of compound **33** with nucleoside **34** to give the desired bisphosphonate analogue **35** (Scheme 11). The latter was found to be a potent inducer of differentiation of K562 erythroid leukemia cells.[38]



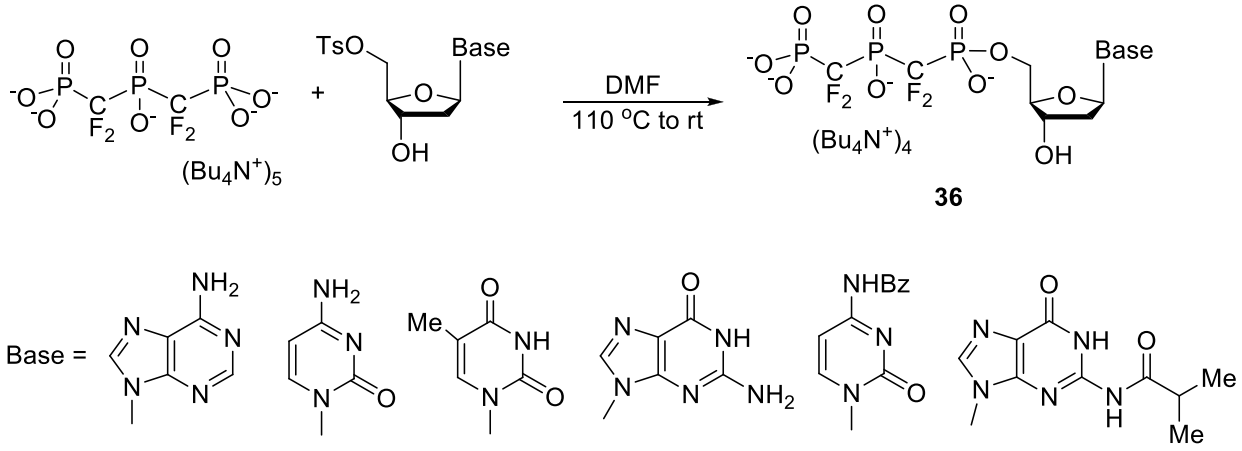
32 **33**



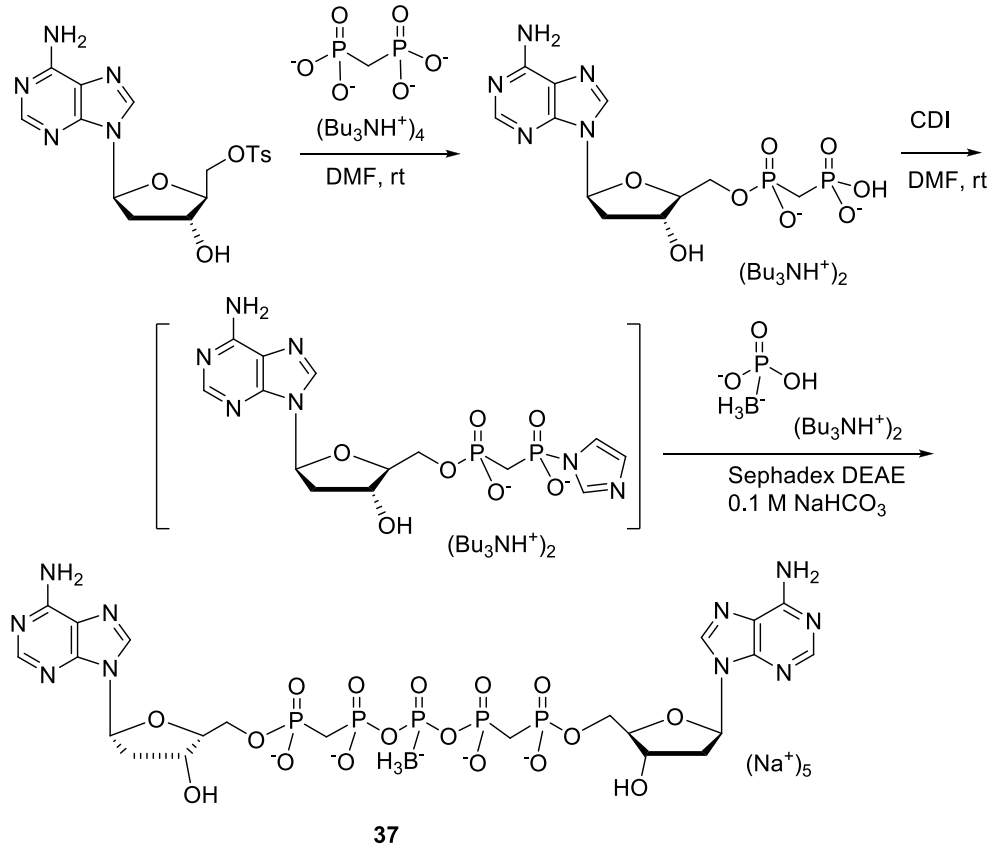
34 **35**

Scheme 11. Synthesis of a nonhydrolyzable CF_2-MBP analogue of thiazole-4-carboxamide and benzamide adenine dinucleotide.

Nucleophilic substitution of tosylate at the 5′-position of a deoxynucleoside was also successfully used for the synthesis of bis-CF_2-substituted deoxynucleotide analogues (Scheme 12). In the cases of deoxyadenosine, deoxythymidine, and protected deoxycytidine, conversions to dNTP analogues **36** were 85%, 91%, and 88%, respectively. However, the protected deoxyguanosine 5′-tosylate reacted with bis(difluoromethylene)-triphosphoric acid very slowly, and the maximum conversion that could be achieved was 10%.[39]



Scheme 12. Preparation of bis-CF_2-substituted deoxynucleotide analogues via nucleophilic substitution of tosylate moiety at the 5′-position of a deoxynucleoside.

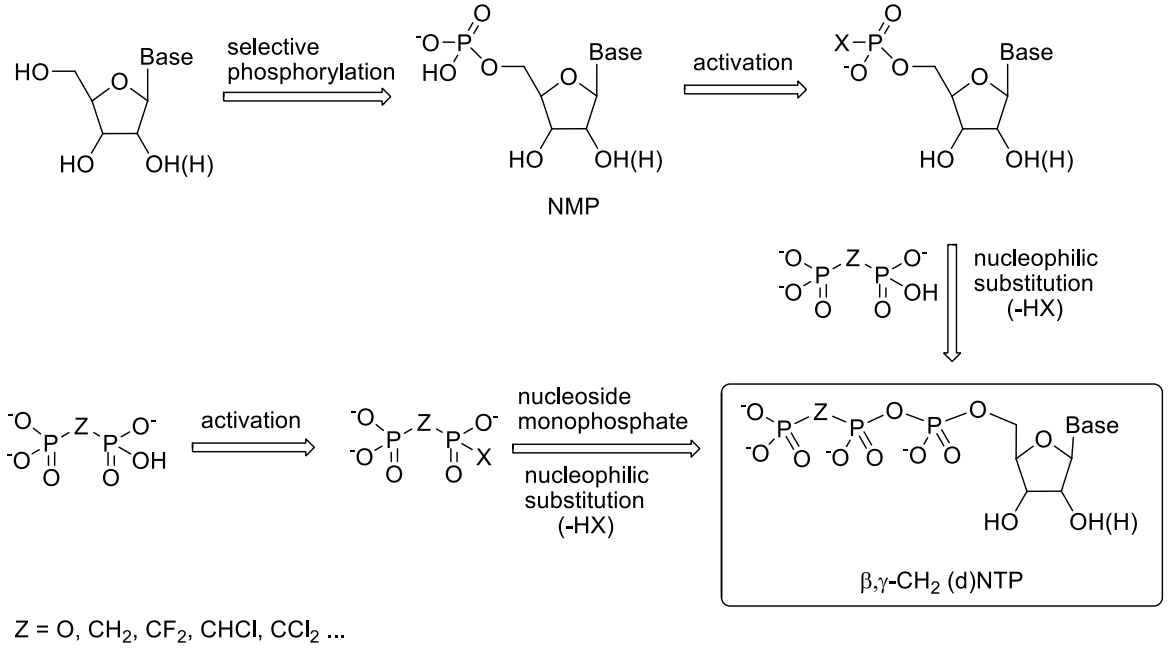


Scheme 13. Synthesis of di-2′-deoxyadenosine α,β:δ,ε-dimethylene-pentaphosphonate.

Using the tosylate substitution and the phosphoroimidazolidate protocol, Eliahu *et al*. synthesized di-2´-deoxyadenosine α,β:δ,ε-dimethylene-pentaphosphonate **37**.[31] The formation of the modified polyphosphate motif required four steps starting from the 5'-*O*-tosyl-2-deoxyadenosine (Scheme 13). Bisphosphonate analogue **37** is the first specific nucleotide pyrophosphatase/phosphodiesterase inhibitor to be described.

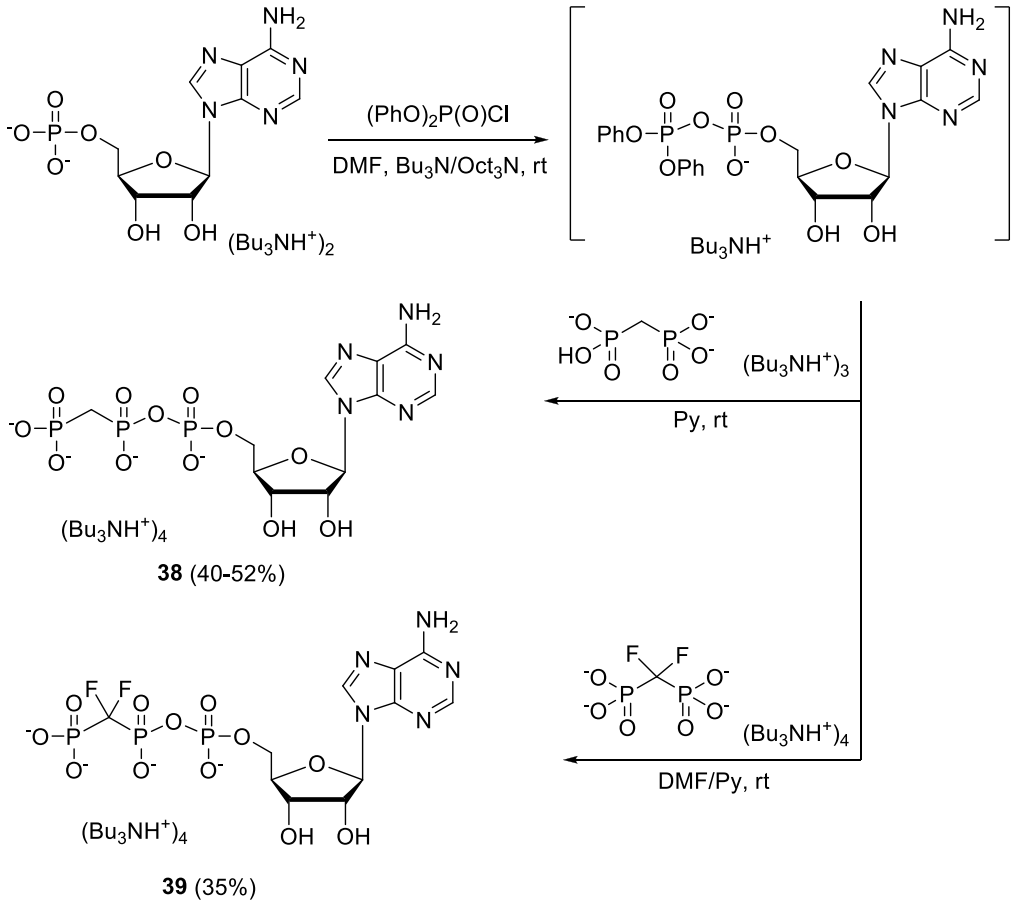
2.2. Synthesis via activated phosph(on)ate substrates

Diphenyl chlorophosphate[40-43], *N*,*N*´-carbonyldiimidazole (CDI)[31,44,45], imidazole/2,2´-dithiopyridine/Ph_3P system[46-50], dicyclohexylcarbodiimide (DCC)[51,52], and trifluoracetic anhydride[53,54] are the most widely used activating reagents for the synthesis of methylene-modified nucleoside tri- and polyphosphates. All coupling methods have the same strategy in common: one nucleotide subunit (usually nucleoside monophosphate or bisphosphonate) is converted via an activation process into an electrophilic substrate and then reacted with a second phosphate or phosphonate subunit acting as a nucleophile. In principle, two alternative approaches can be used for triphosphate bridge formation. In the first, a nucleotide is activated at the stage of monophosphate and then coupled to a bisphosphonate. In the second, a bisphosphonate is activated and coupled with a nucleoside monophosphate. Thus, the reaction sequences shown in Scheme 14 were the basis of numerous works in which nucleotide 5´-(β,γ-methylene) triphosphates were prepared via activated phosphate/phosphonate substrates.[55]



Scheme 14. Simplified general approach for the synthesis of β,γ-methylene nucleoside triphosphates via phosphate/phosphonate activation.

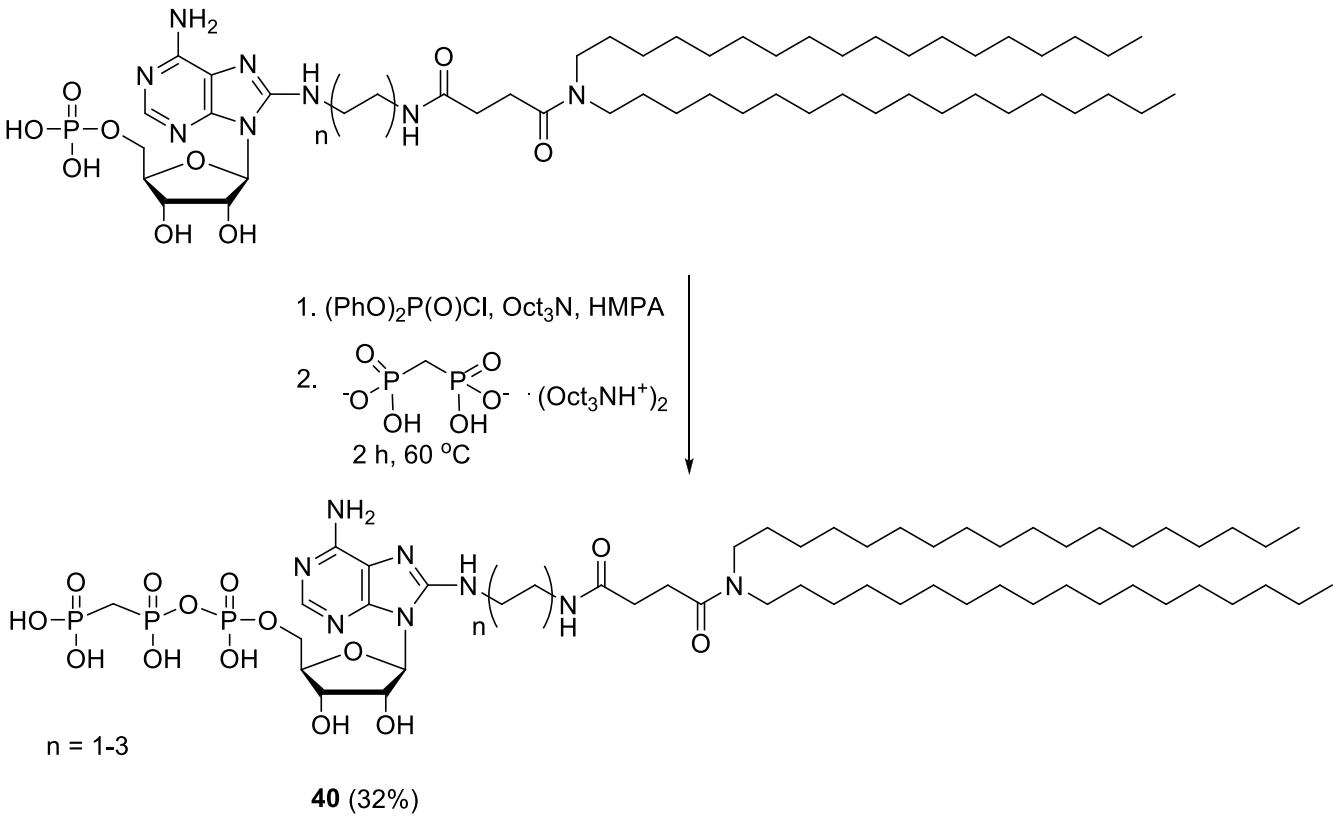
A simple, one-pot method using the diphenyl chlorophosphate technique to prepare β,γ-modified ATP analogues **38** and **39** is illustrated in Scheme 15.[40,56] The reactions proceed at room temperature in Py or Py/DMF solution to give the corresponding products in moderate yields. This approach was the most successful to obtain solid-supported 5´-(α-*P*-thio) triphosphate oligonucleotide analogues compared to other methods involving the use of phosphoroamidate or salicyl-phosphite intermediates.[41]



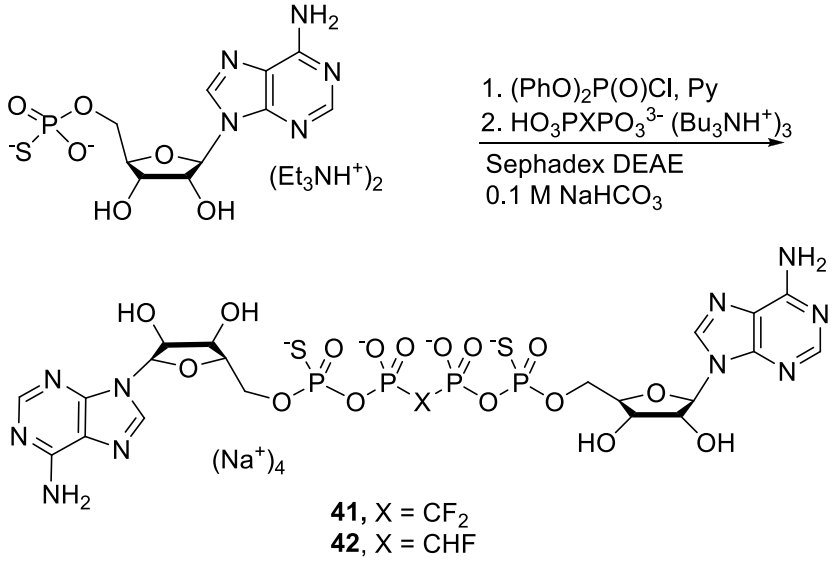
Scheme 15. Synthesis of β,γ-CH_2- and β,γ-CF_2 ATP analogues via $(PhO)_2P(O)$-activated AMP.

Schmitt and Tampé reported an example for the application of diphenyl chlorophosphate activation in a late step of the synthesis of a novel class of nonhydrolyzable ATP-lipids **40** where the nucleotides are covalently attached via C^8 (or N^6)-position of the adenine ring to a synthetic lipid (Scheme 16).[42] Possible applications of the novel class of ATP-lipid have been discussed.

Diphenyl phosphorochloridate activation is also applicable for the construction of dinucleotide analogues. Thus, the procedure shown in Scheme 17 demonstrates the most simple and effective synthesis of P^1,P^4-dithio-P^2,P^3-CF_2- and CHF-analogues of diadenosyl 5′,5‴-P^1,P^4-tetraphosphate (Ap_4A). In the case of the $AP_sPCHFPP_sA$ analogue **42**, four diastereomers are formed in equal proportions. Details of separation of these diastereomeric species and their biological application have been described.[57]



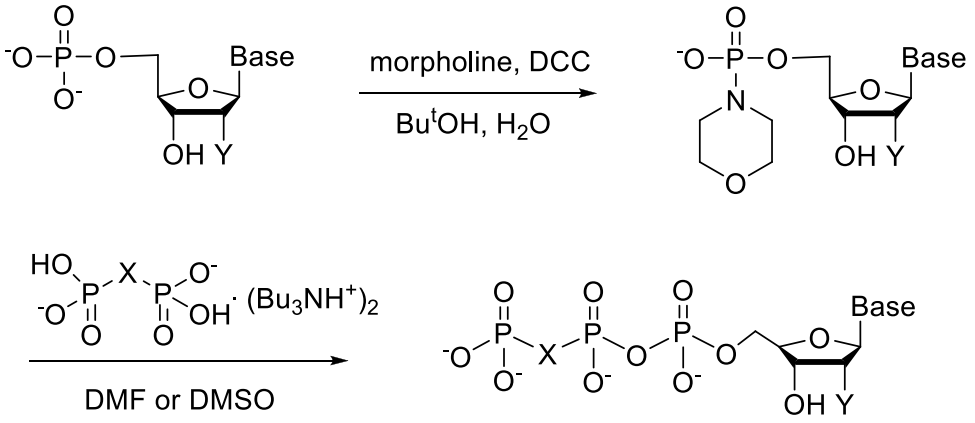
Scheme 16. Application of $(PhO)_2P(O)Cl$-activation in the synthesis of C^8-modified nonhydrolyzable ATP-lipids.



Scheme 17. Preparation of P^1,P^4-dithio-P^2,P^3-CF_2- and CHF-analogues of diadenosyl 5',5'''-P^1,P^4-tetraphosphate.

Currently, the most commonly used methods for the preparation of β,γ-methylene-modified nucleotides involve either a morpholidate or imidazolidate activation. The morpholidate method, introduced by Khorana as one of the first successful strategies for the synthesis of nucleoside-5´-polyphosphates,[58] employs a two-step process which involves conversion of nucleoside monophosphate (NMP) to the corresponding

morpholidate via dicyclohexylcarbodiimide (DCC) activation followed by conjugation with the appropriate bisphosphonate. Myers prepared the first bisphosphonate analogue of adenosine 5′-triphosphate, β,γ-CH_2 ATP, by condensing methylenebisphosphonic acid with adenosine 5′-phosphoromorpholidate.[59] Similar analogues of ATP, GTP, UTP and CTP having β,γ-CF_2, CCl_2, and CFH groups have been described (Scheme 18).[52,60-62]



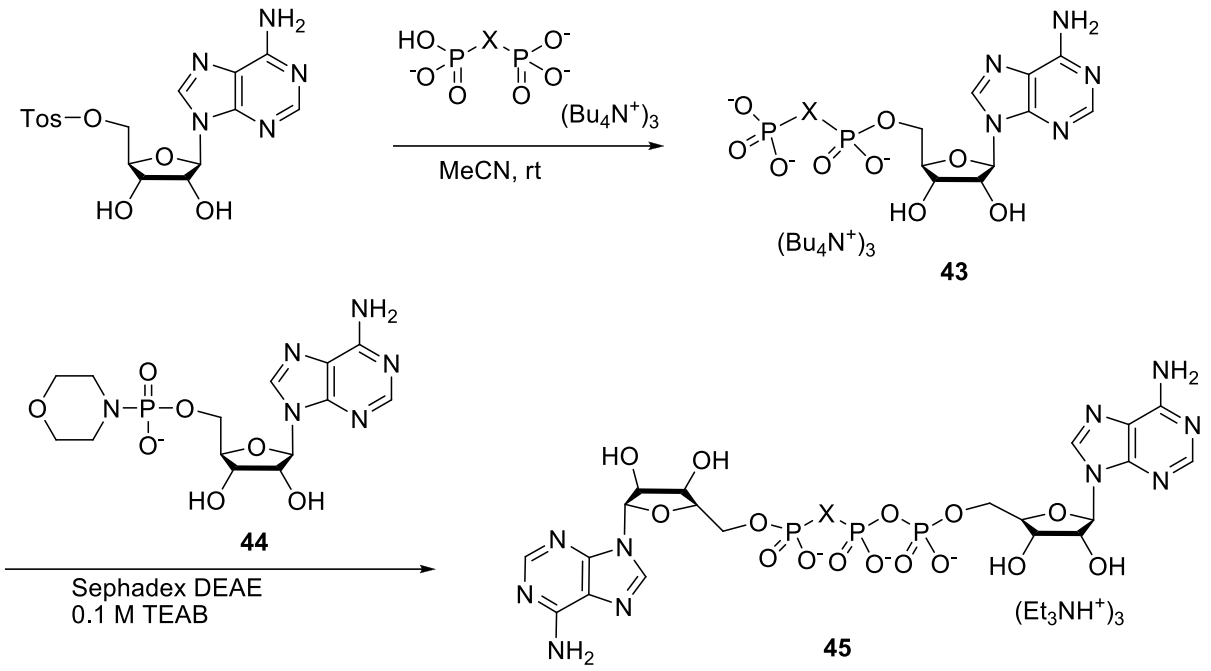
Base = adenine, guanine, cytosine
X = CH_2, CF_2, CHF, CCl_2, CFCl, CBr_2, CHBr, CMe_2, CHMe, CFMe
Y = H, OH

Scheme 18. Synthesis of β,γ-methylene bisphosphonate (d)NTP analogues via morpholidate intermediates.

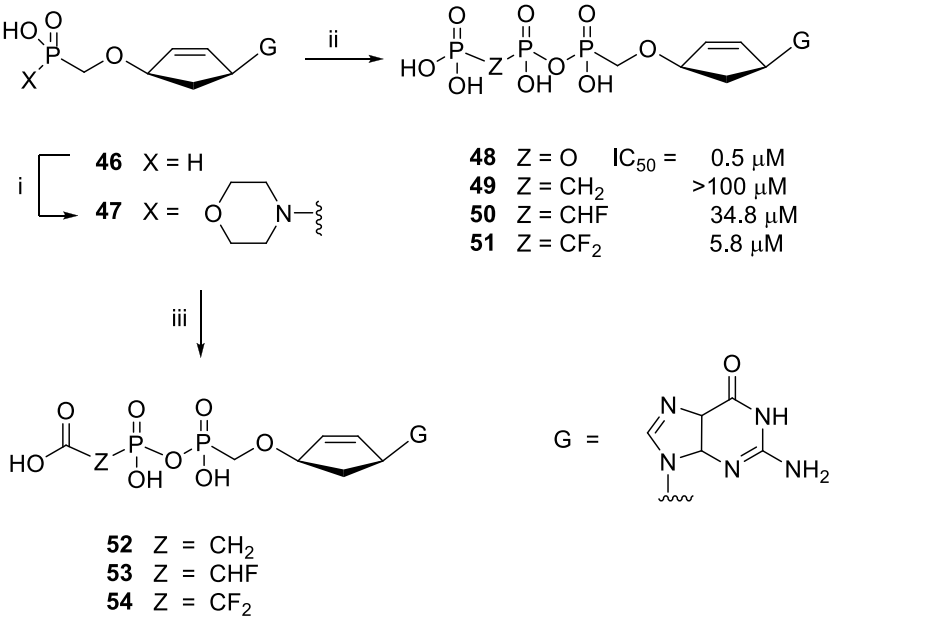
The effective route to analogues of diadenosine 5′,5‴-*P*^{1}, *P*^{3}-triphosphate **45** involves the condensation of adenosine *C*-phosphoromorpholidate **44** with *P*^{1}, *P*^{2}-methylene analogues of ADP **43**, conveniently prepared by the method (Scheme 19).[37]

The search for carbocyclic nucleotides with potent anti-HIV activity led to the synthesis of the pyrophosphoryl phosphonate **48**[63,64] and its diphosphonate analogues **49**[65,66] with progressive fluoro-substitution within the βγ-methylene linker group as shown in Scheme 20.

Noteworthy features of the chemical syntheses include the transformation of the nucleoside monophosphate **46** into the activated morpholidate **47**, and the coupling of **47** with the corresponding bisphosphonate. Nucleotides **50, 51** were found to be potent inhibitors of HIV reverse transcriptase. The three nucleotide triphosphate mimics **52-54** were also synthesized and tested as inhibitors of HIV RT in an enzyme assay. Both **52** and the monofluorinated analogue **53** showed relatively poor activity, being three orders of magnitude less active than the parent compound **48**. The difluorinated analogue **54** was markedly more effective than the monofluorinated substrate but was still two hundred times less potent than **48**. The disappointing activity of **52-54** may be due to the fact that the carboxy group is a poor mimic of the terminal phosphonate group in compound **51**.[66]



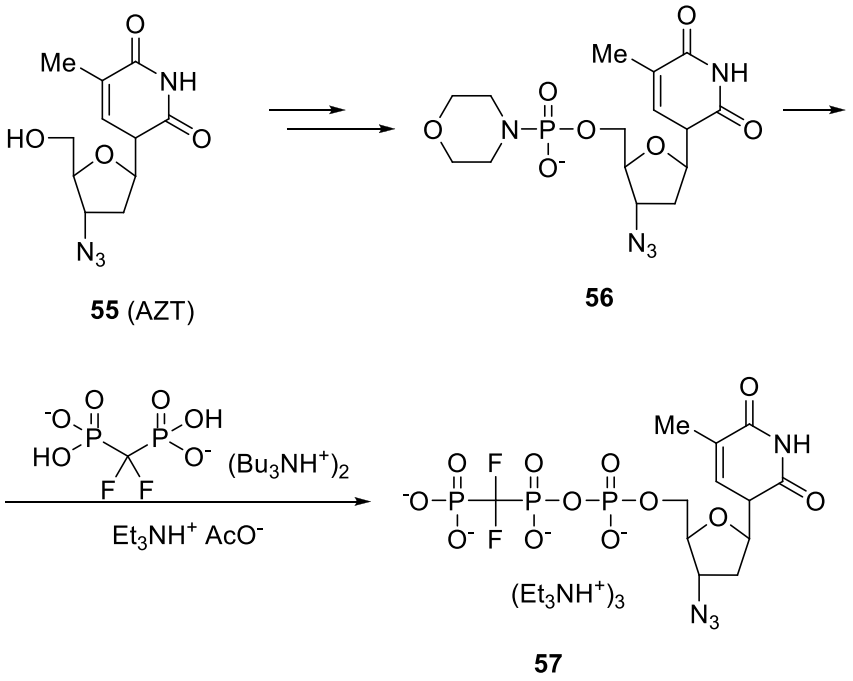
Scheme 19. Synthesis of analogues of diadenosine 5′,5‴-*P*^{1}, *P*^{3}-triphosphate utilizing the combination of the Poulter method and morpholidate activation.



Reagents and conditions: i, morpholine, DCC, ^{t}BuOH, H_2O; ii, $[(HO)_2P(O)]_2Z \cdot Bu_3N$ (Z = CH_2, CHF, CF_2), DMSO, rt; iii, $(HO)_2P(O)ZCO_2H$ (NBu_3 salt), DMSO, rt

Scheme 20. Preparation of carbocyclic nucleotide analogues with progressive fluoro-substitution within the β,γ-methylene linker group.

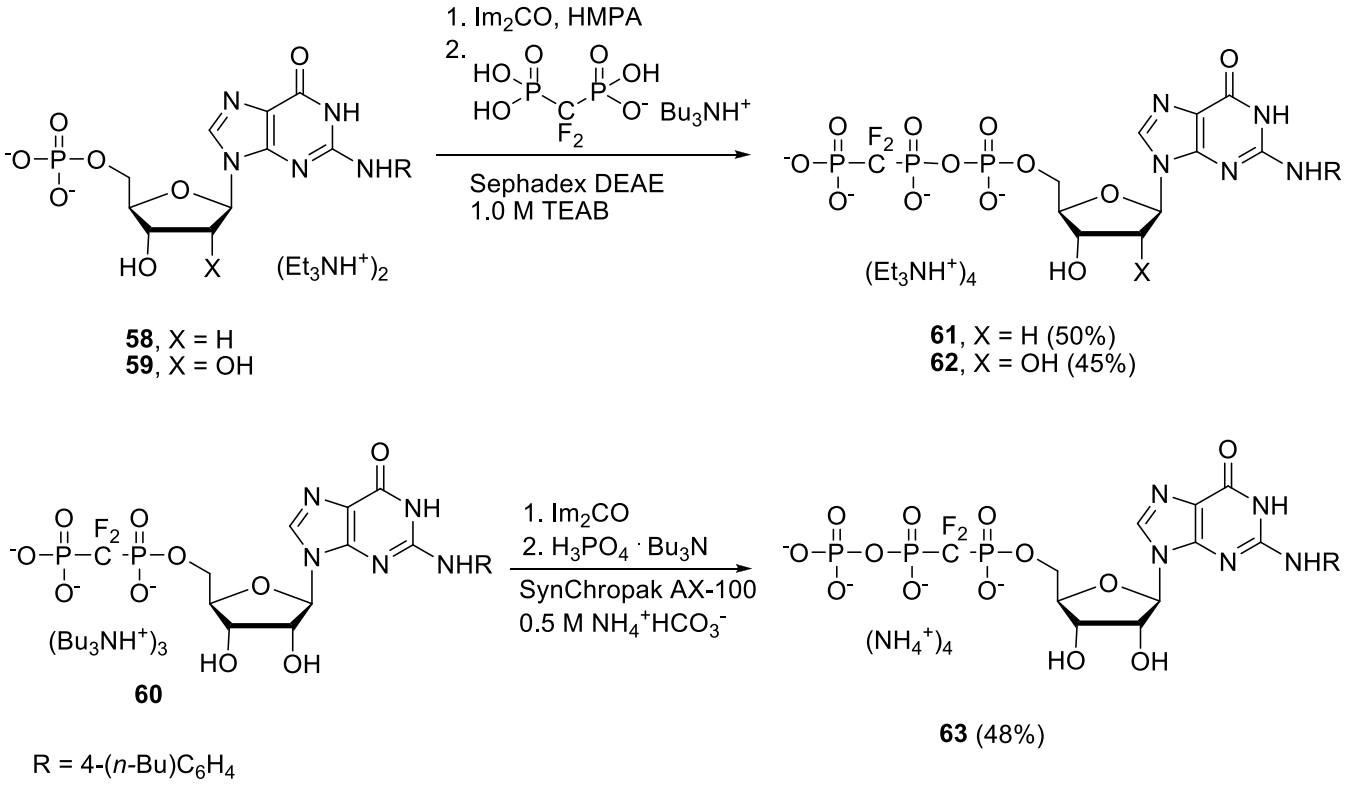
3′-Azido-3′-deoxythymidine (**55**, AZT), used extensively as an approach to the management of HIV infection, is metabolized in cells to the corresponding 5′-triphosphate (AZTTP).[67] The preparation of the β,γ-CF_2-bridged analogue of AZTTP **57** was accomplished by the coupling of difluoromethylenebisphosphonic acid to 3′-azido-3′-deoxythymidine 5′-monophosphate, activated as the morpholidate **56** (Scheme 21). The difluoromethylene analogue **57** was 30-fold less effective than AZTTP as a competitive inhibitor of HIV-1 RT but 10-fold more effective than the methylenephosphonate analogue β,γ-CH_2-AZTTP.[43]



Scheme 21. Synthesis of the the β,γ-CF_2 -bridged analogue of AZTTP.

Phosphorimidazolidate intermediates result from activation of nucleoside monophosphates with 1,1′-carbonyldiimidazole (CDI). Hoard and Ott's original research on this transformation featured syntheses of triphosphates from dNMPs and inorganic pyrophosphate.[68] A similar approach is applicable to the synthesis of β,γ-methylene nucleoside triphosphates. Addition of acidic bisphosphonate activates the phosphorimidazolidate toward nucleophilic displacement giving phosphonate-modified NTP. The imidazolidate method is usually performed as a one-pot synthesis; the nucleoside monophosphate is converted to the imidazolidate by activation with CDI followed by the addition of the appropriate bisphosphonate.[31,44,45] Complications in the imidazolidate procedure have been reported when ribonucleosides with unprotected vicinal diols were activated with CDI. This phosgene equivalent easily forms cyclic carbonates that were carried through as impurities in the triphosphorylation procedure. Additionally, nucleoside phosphorimidazolidate can react sluggishly with methylenebisphosphonate, requiring prolonged reaction times or the use of catalysts such as $ZnCl_2$ or $CdCl_2$.[48,69] In some cases, reversal of the commonly used strategy, such that the α,β-methylene NDP is the nucleophile and the phosphorimidazolidate is the electrophile, resulting in a high yield of the desired NTP analogue without the need for catalyst or long reaction time.

Wright and co-workers reported their studies on DNA pol α and β inhibition by CF_2-substituted (d)NTP analogues bearing a 4-(*n*-Bu)C_6H_4-group attached to the base residue (Scheme 22).[29] 5′-[β,γ-CF_2 triphosphates] **61** and **62** were synthesized by reaction of the corresponding 5′-phosphates **58** and **59**, activated by 1,1′-carbonyldiimidazole, with the soluble tri-*n*-butylammonium salt of difluoromethylenebisphosphonic acid.

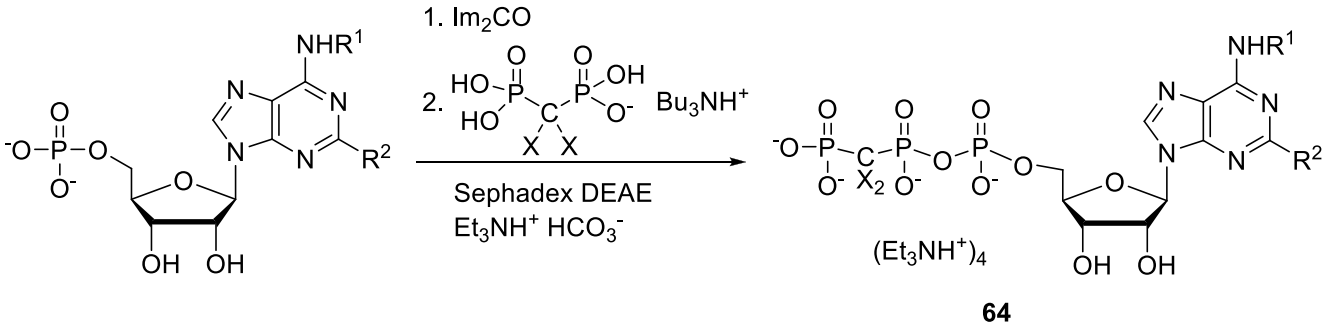


Scheme 22. Preparation of CF_2-bridged polyphosphate analogues **61**-**63** via phosphorimidazolidate protocol.

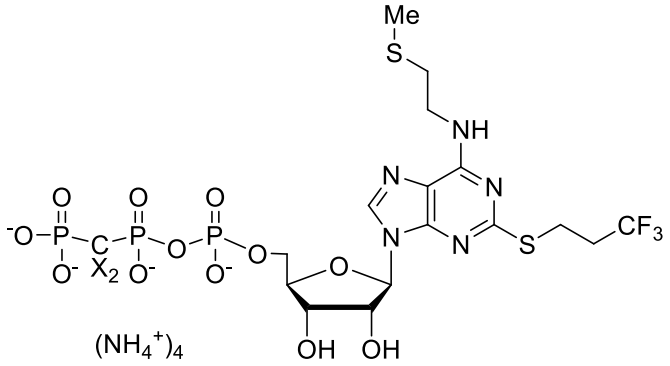
Preparation of the α,β-CF_2 derivative of N^2-(4 -butylphenyl)guanosine 5′-triphosphate, compound **63**, required first the synthesis of the bisphosphonate **60**. The latter was prepared by treatment of a protected 5′-tosyl nucleoside with difluoromethylenebisphosphonate, followed by deprotection. Condensation of this nucleotide, activated with 1,1′-carbonyldiimidazole, with orthophosphate gave α,β-CF_2 triphosphate **63**. The phosphonates were tested for their ability to displace [^{3}H]GDP from GTP binding protein cellular (EC) and oncogenic (Leu-61) Ha-*ras* p21, and for their ability to inhibit DNA polymerase from Chinese hamster ovary cells. (β,γ)-CF_2 4-(*n*-Bu)dGTP analogue **61** was reported to inhibit DNA pol α with K_i = 0.007 μM, whereas regular 4-(*n*-Bu)dGTP has K_i = 0.005 μM.

Ingall *et al*. reported the synthesis of a series of ATP analogues **64** designed to act as antithrombotic agents. Substitution of the adenine moiety enhanced affinity and selectivity for the P_{2T} receptor and led to the development of a highly potent compound **64a** with IC_{50}=0.4 nM. The whole series were prepared via phosphorimidazolidate protocol (Scheme 23).[70]

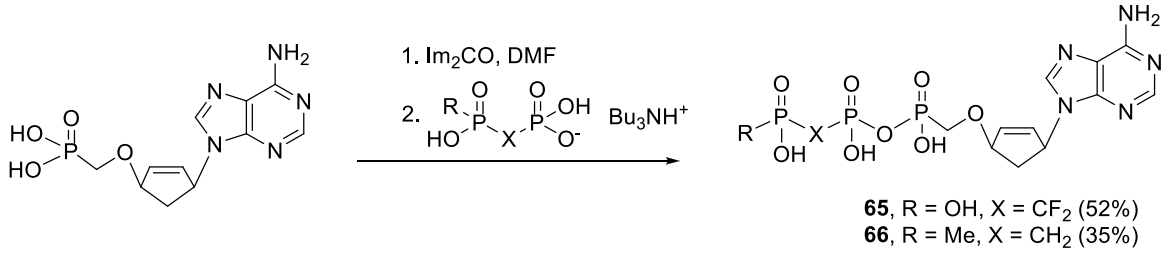
Nucleotide analogues modified at the glycone and all three phosphate residues were reported by Roberts and co-workers as highly stable in human blood serum with half-lives toward hydrolysis up to 4.5 days.[71] These analogues were shown to be selective inhibitors of DNA synthesis, catalyzed by retroviral reverse transcriptases and terminal deoxynucleotidyl transferazes. A typical synthetic procedure is shown for ATP analogues **65** and **66** in Scheme 24.



R^1	R^2	X	Yield (%)
H	SEt	Cl	9
H	SPr	F	5
H	SPr	Cl	7
CH_2CF_3	SPr	Cl	12
CH_2CH_2OMe	SPr	Cl	6
CH_2CH_2SMe	SPr	Cl	6
H	$SCH_2CH_2CF_3$	Cl	21
CH_2CF_3	$SCH_2CH_2CF_3$	Cl	4
CH_2CH_2OMe	$SCH_2CH_2CF_3$	Cl	10
CH_2CH_2SMe	$SCH_2CH_2CF_3$	Cl	4



Scheme 23. Synthesis of antithrombotic nucleotide analogues via phosphorimidazolidate protocol.

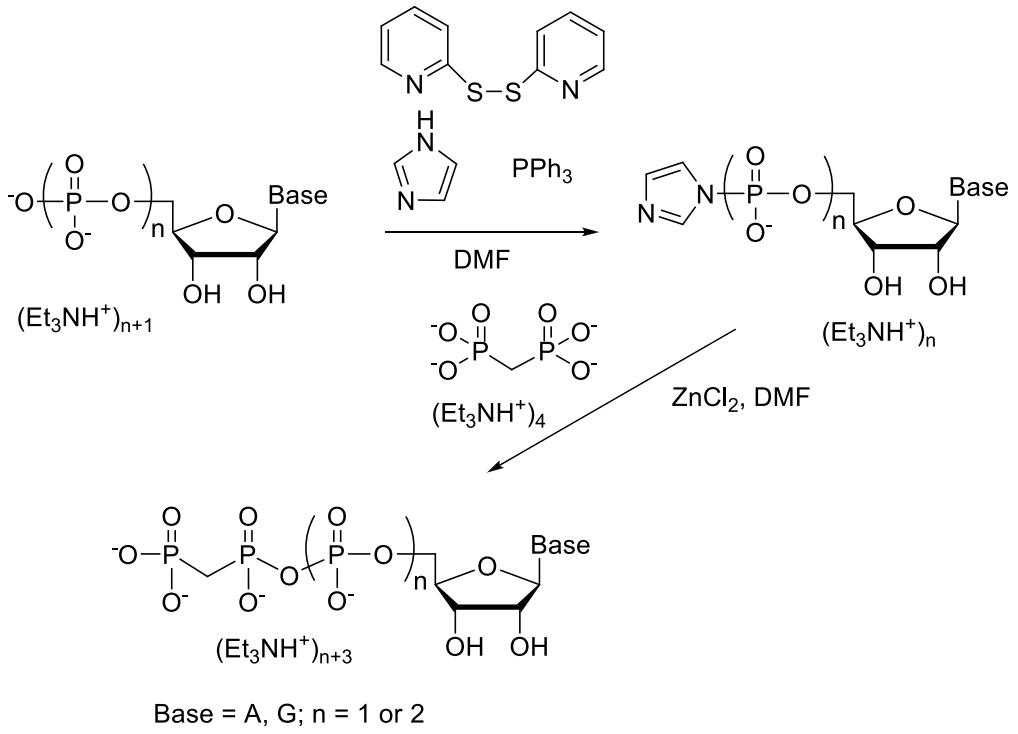


Scheme 24. Synthesis of glycone and triphosphate modified nucleotide analogues.

The special need for nucleotides with a modified polyphosphate chain as rapid and highly efficient coupling reagents led to the development of an effective method of preparation of phosphorimidazolidate intermediates via the Mukaiyama-Hashimoto oxidation-reduction conditions.[72]

Scheme 25 illustrates the synthetic pathway for obtaining methylene analogue of nucleoside tri- and tetraphosphates. The reaction was performed by activation of a nucleotide unit with imidazole in the presence of triphenylphosphine/2,2´-dithiodipyridine (DTDP) system, followed by coupling with organic salt of bisphosphonate carried out in DMF in a presence of an 8-fold excess of $ZnCl_2$.[69] A similar approach has been successfully exploited for the synthesis of mono- and dinucleoside di-, tri-, tetra- and even penta-phosphates and their methylene analogues.[46-50] The coupling reactions occurred efficiently without significant

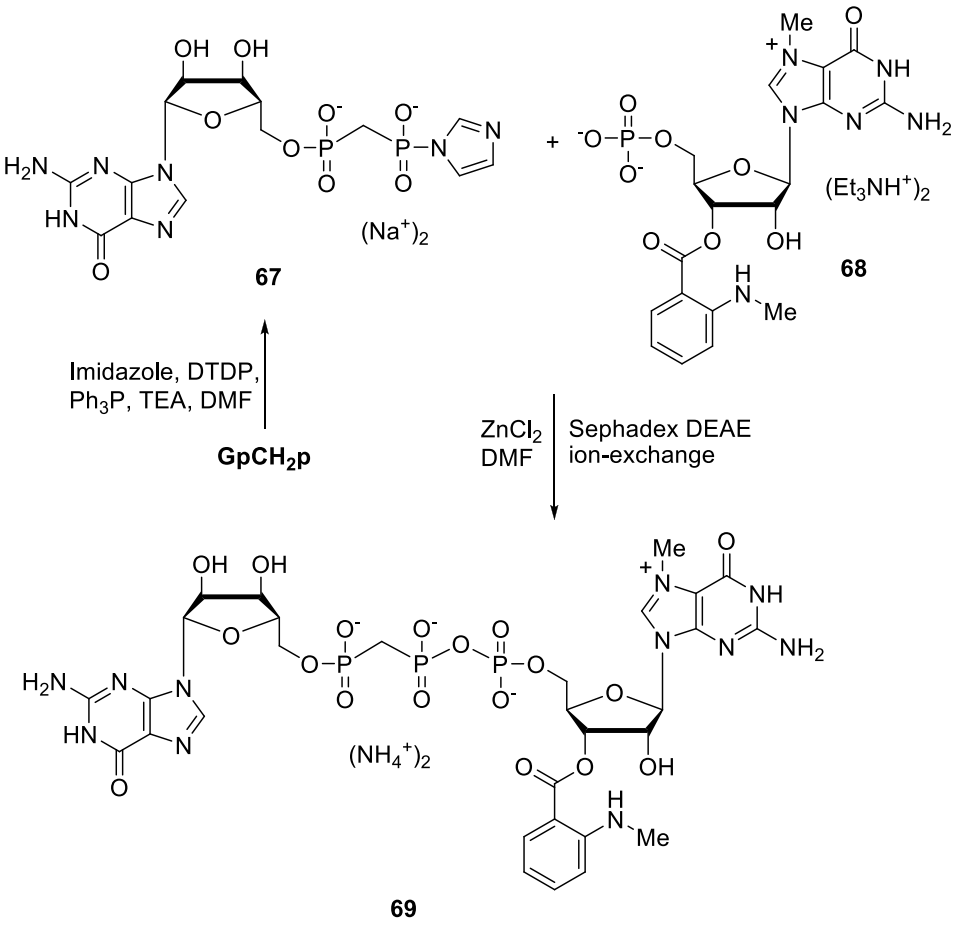
accumulation of by-products which is important because of purification difficulties common for this class of compounds.



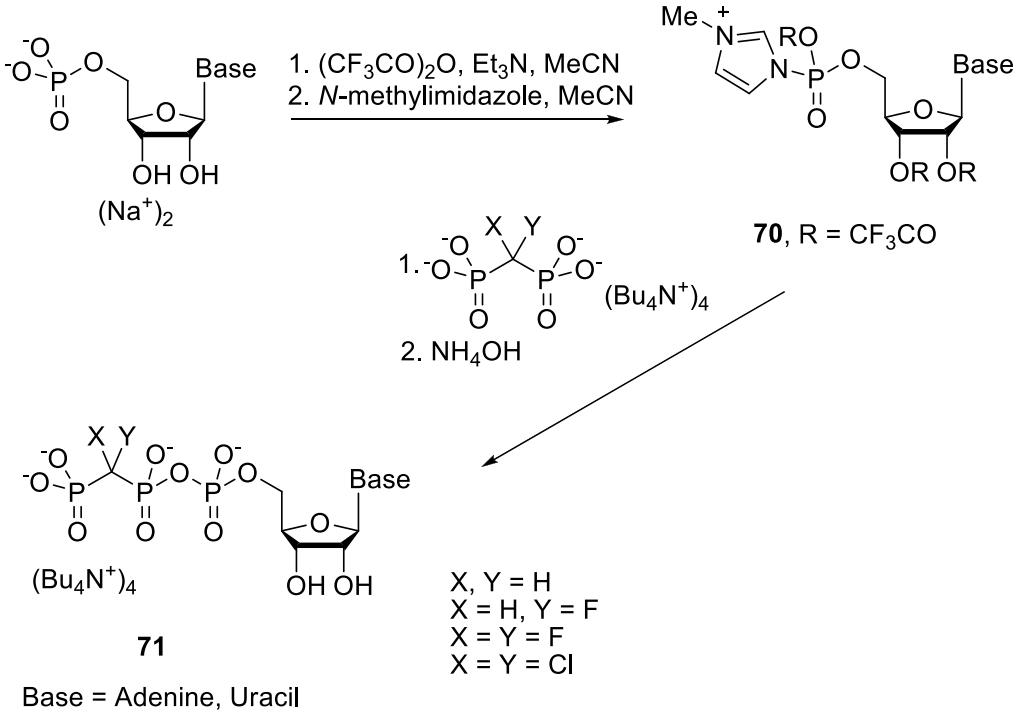
Scheme 25. Synthesis of CH_2-modified nucleotide analogues via the Mukaiyama-Hashimoto oxidation-reduction conditions.

In order to obtain dinucletide cap analogues labeled at the ribose of the 7-methylguanosine moiety with *N*-methylanthraniloyl, Jemielity and co-workers have used the reverse strategy involving $ZnCl_2$-mediated coupling of bisphosphonate-modified nucleotide *P*-imidazolidate **67** with fluorescently labeled nucleoside monophosphate **68** (Scheme 26).[73] Compound **69** was obtained with a yield of 12% after two purification steps, ion-exchange chromatography and HPLC.

A modified imidazolidate approach to nucleoside 5′-β,γ-chloromethylenetriphosphates is shown in Scheme 27. The electrophilic 5′-monophosphate-*N*-methylimidazolidates **70** were formed by the reaction of nucleoside 5′-monophosphates with an excess of trifluoroacetic anhydride in the presence of triethylamine followed by treatment with *N*-methylimidazole. The intermediate **70** was then added to the bisphosphonate, thus affording the corresponding nucleoside triphosphate analogue **71** in a reproducible and efficient manner (>72% isolated yield).[54]

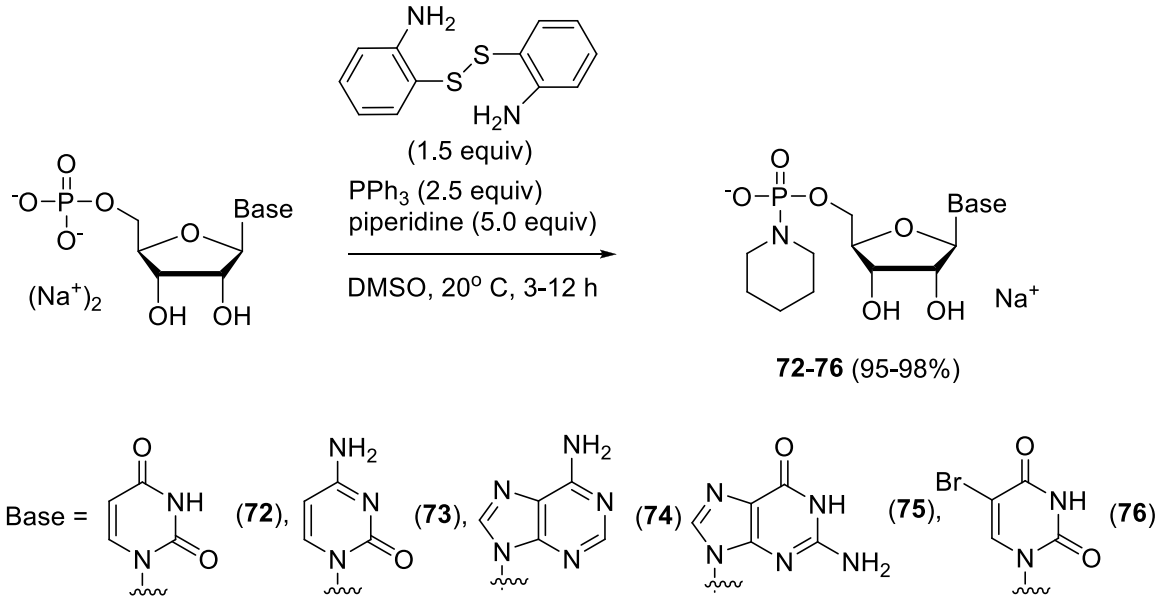


Scheme 26. Synthesis of CH_2-modified triphosphate dinucleotide cap analogue **69**.



Scheme 27. Synthesis of nucleoside 5′-β,γ-methylenetriphosphates from electrophilic 5′-monophosphate -*N*-methylimidazolidates.

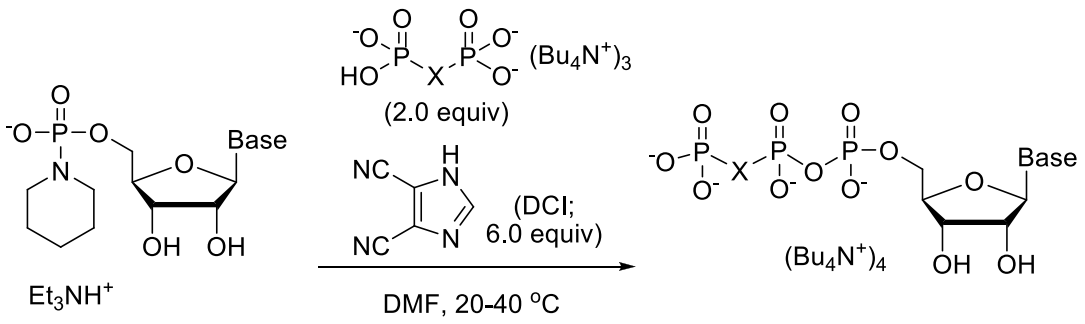
Recently, Sun and co-workers have developed a novel P^V-N activation method for the synthesis of nucleoside 5′-triphosphates and their β,γ-bridging oxygen-modified analogues from nucleoside 5′-phosphoropiperidates with 4,5-dicyanoimidazole (DCI) as the activator.[74] A high-yielding and chromatography-free method for preparation of nucleoside 5′-phosphoropiperidates **72**-**76** is shown in Scheme 28.



Scheme 28. Method for synthesis of nucleoside 5'-phosphoropiperidates.

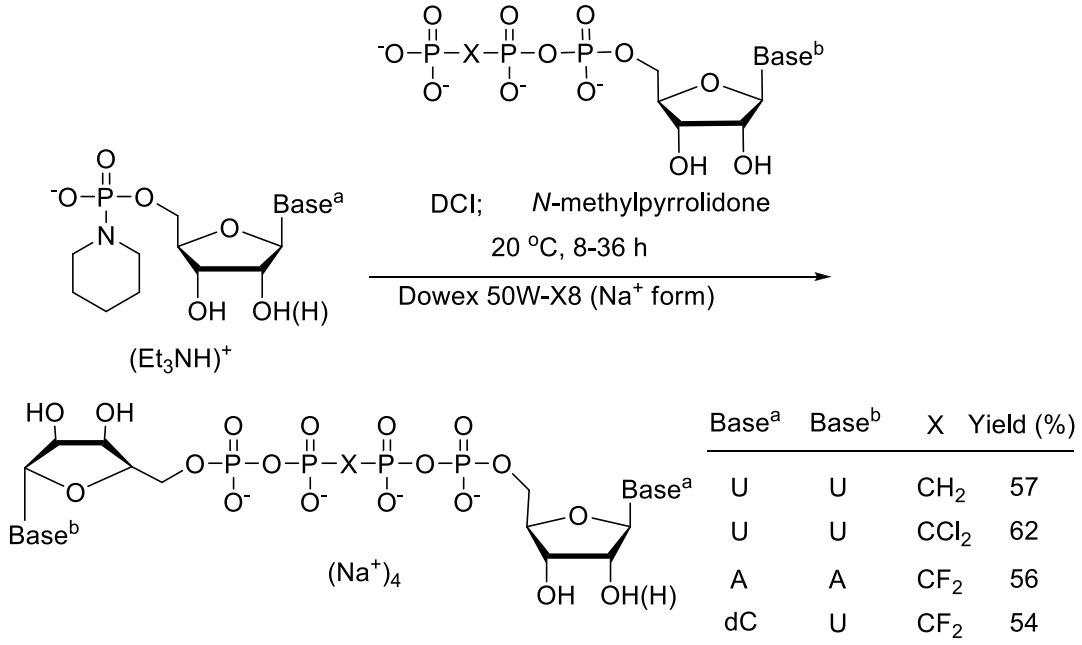
The obtained nucleoside 5′-phosphoropiperidates exhibited excellent reactivity toward bisphosphonate reagents in the presence of 4,5-dicyanoimidazole and afforded β,γ-CX_2-NTP products in high isolated yields (Scheme 29). In the following research, the same authors extended the application of the phosphoropiperidate/DCI system for the preparation of symmetrical and asymmetrical P^2,P^3-CX_2-dinucleoside tetraphosphates (Scheme 30). Compared to the conventional phosphoromorpholidate method, this approach afforded products in shorter reaction time and higher isolated yields.[75] A one-pot method for DCI-promoted synthesis of symmetrical NppCX_2ppN bisphosphonate analogues simply from nucleoside 5′-phosphoro-piperidates without using nucleoside phosphonates has also been described.[76]

A very effective synthetic procedure for the preparation of a series of dinucleoside tetraphosphate analogues via activated bisphosphonates and nucleoside monophosphates was developed by Yanachkov *et al*.[77] They found that organic salts of pyrophosphoric acid and its halomethylenebisphosphonate analogues react with an excess of 1,1′-carbonyldiimidazole (CDI) to give stable, isolable diimidazolidates **77**, and that these diimidazolidates react with nucleoside 5′-monophosphates or monothiophosphate **78** to give the corresponding nucleotide analogues **79** conveniently and in high yield (Scheme 31). Several bisphosphonate analogues of P^1,P^4-di(adenosine-5′) tetraphosphate were evaluated with respect to their effects on platelet aggregation and function of the platelet $P2Y_1$, $P2Y_{12}$, and P2X1 receptors. Some of the compounds showed very potent (nanomolecular level) inhibition of ADP induced human platelet aggregation, thus presenting a new and promising class of antiplatelet drugs (Figure 1).[78]



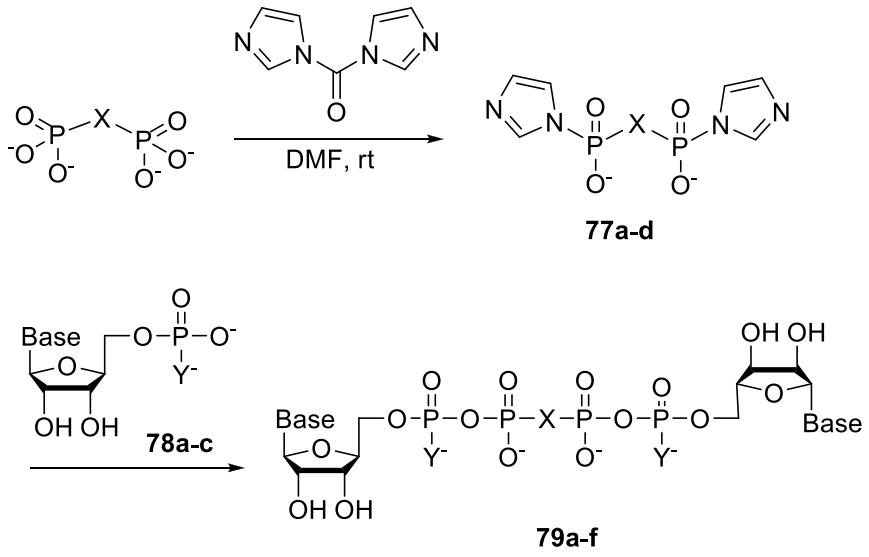
X	Base	Yield (%)	X	Base	Yield (%)
CH_2	U	79	CH_2	5-BrU	78
CH_2	C	71	CF_2	U	71
CH_2	A	72	CCl_2	U	78
CH_2	G	74	CBr_2	U	84

Scheme 29. DCI promoted conversion of nucleoside 5´-phosphoropiperidates to β,γ-CX_2 NTPs.



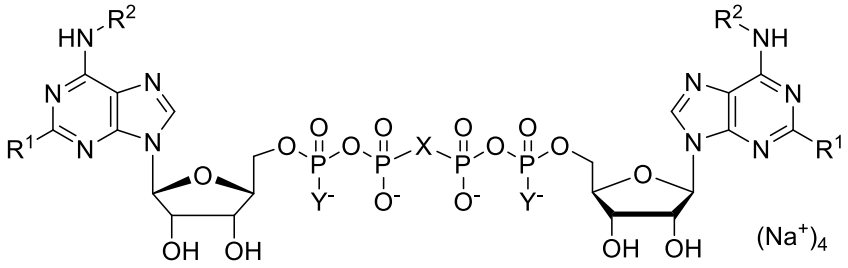
Base[a]	Base[b]	X	Yield (%)
U	U	CH_2	57
U	U	CCl_2	62
A	A	CF_2	56
dC	U	CF_2	54

Scheme 30. DCI promoted synthesis of P^2,P^3-CX_2 dinucleoside tetraphosphates.



77: X = O (a); CHCl (b); CHF (c); CCl_2 (d)
78: Base = A, Y = O (a); Base = A, Y = S (b); Base = U, Y = O (c)
79: Base = A, X = Y = O (a); Base = A, X = O, Y = S (b); Base = A, X = CHCl, Y = O (c);
Base = A, X = CHCl, Y = S (d); Base = A, X = CHF, Y = O (e); Base = U, X = Y = O (f)

Scheme 31. Synthesis of bis-imidazolidates of pyrophosphoric and halomethylenebisphosphonic acids and their use in the synthesis of dinucleoside tetraphosphonate analogues.



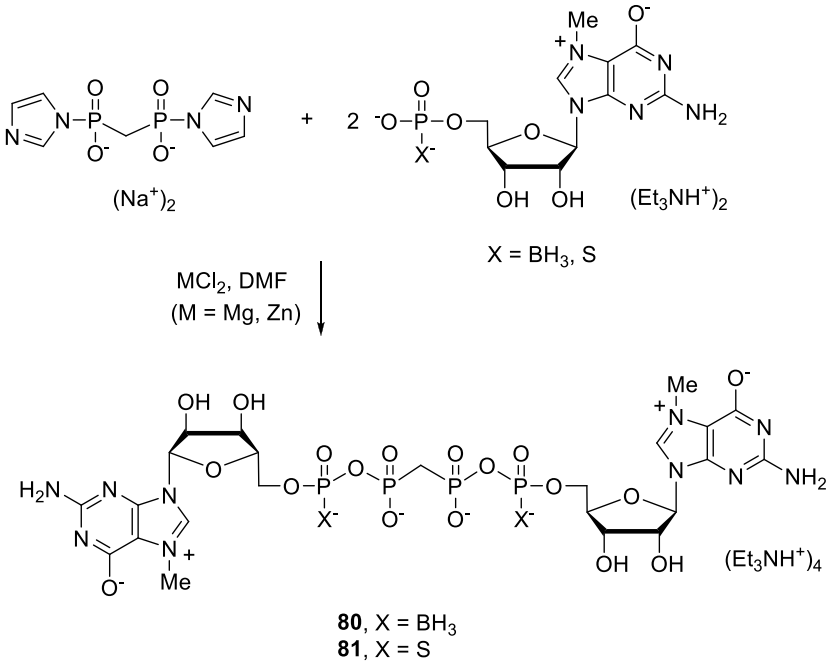
R^1	R^2	X	Y
H	H	CHCl	S
MeS	H	CHCl	O
MeS	H	CHCl	S
EtS	H	CHCl	S
PrS	H	CHCl	S
$CF_3(CH_2)_2S$	H	CHCl	S
n-$C_5H_{11}S$	H	CHCl	S

R^1	R^2	X	Y
Cl	H	CHCl	S
Br	H	CHCl	S
I	H	CHCl	S
H	Me	CHCl	S
H	Pr	CHCl	S
MeS	Me	CHCl	S
MeS	H	CCl_2	S

Figure 1. Structures of bisphosphonate Ap_4A analogues presenting a new promising class of antiplatelet drugs.

The activation strategy in which a bisphosphonate is activated with imidazole and then coupled with a non-activated nucleotide has been applied by Polish researchers to the synthesis of di-(7-methylguanosine)-

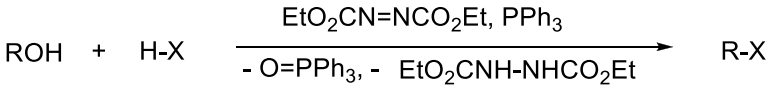
tetraphosphates and their α,δ-diborano and α,δ-dithio analogues **80**, **81** containing a β,γ-methylene group (Scheme 32).[79]



Scheme 32. Synthesis of dinucleotide cap analogues modified within a polyphosphate chain.

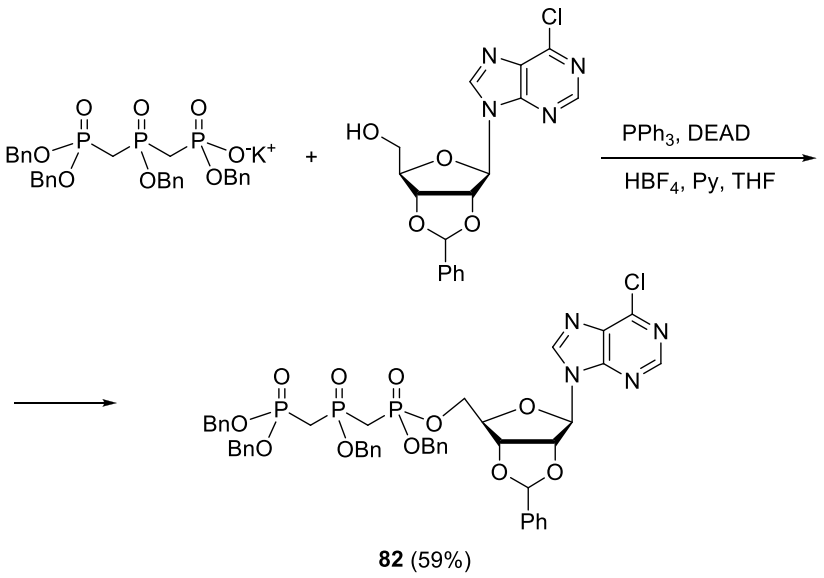
2.3. Synthesis via the Mitsunobu reaction

Discovered in 1967 by Oyo Mitsunobu, this mild multicomponent reaction permits esterification of an acidic component (HX, pK_a < 11) with a primary or a secondary alcohol (ROH) in the presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) (Scheme 33).[80,81]



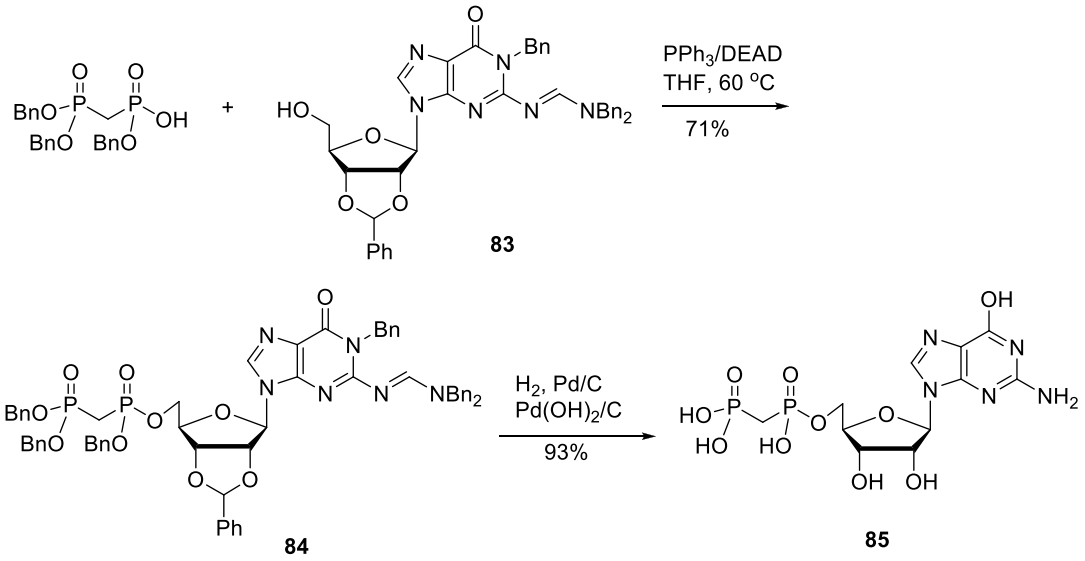
Scheme 33. The Mitsunobu reaction

Coupling of nucleosides with phosphoric or phosphonic acids using the Mitsunobu reaction, pioneered by Mioskowski and co-workers,[82] has become one of the important methods available for the synthesis of nucleoside polyphosphates and polyphosphonates. The earliest report of the effectiveness of the Mitsunobu condensation in nucleoside phosphonate chemistry was the preparation of 6-chloroadenosine α,β:β,γ-bismethylenetriphosphate analogue **82** (Scheme 34).[83] The original procedure involves treatment of phosphonic acid salt (1 equiv), nucleoside (1 equiv), and triphenylphosphine (3 equiv) in anhydrous pyridine with HBF_4 (1 equiv) followed by dropwise addition of DEAD (3 equiv).



Scheme 34. Synthesis of a 6-chloroadenosine α,β:β,γ-bismethylenetriphosphate analogue.

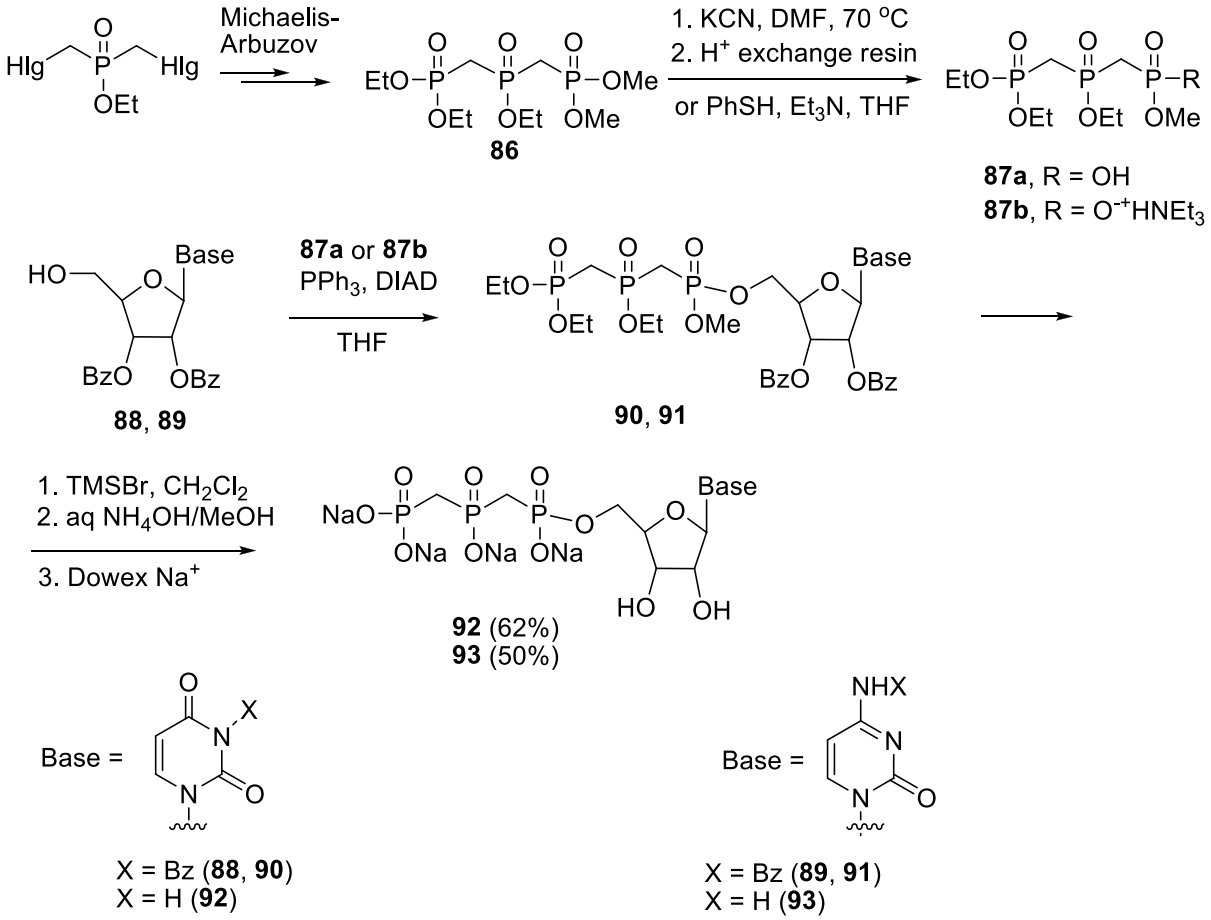
The same strategy, namely the Mitsunobu condensation of tribenzyl methylenebisphosphonate with protected guanosine **83**, has been utilized for the synthesis of nucleoside bisphosphonate **85**. Hydrogenolysis of compound **84** was achieved using a mixture of Pd/C and Pearlman catalyst[84] (Scheme 35).



Scheme 35. Synthesis of 9-[5′-*O*-(methylenebisphosphonate)-β-D-ribofuranosyl]guanine.

An attractive feature of Mitsunobu's phosphorylation is the good or high yield of products for some nucleoside substrates. Disadvantages of the method are a lack of tolerance to purine bases and the difficulties that may be encountered in the synthesis of the suitable methylene phosphate analogues. Thus, if adenosine or guanosine is used as substrates for the Mitsunobu reaction the yields of the coupling are usually lower due to intramolecular nucleophilic attack of the activated 5′-position by a N^3 atom of the base.[82-85] Another possible side reaction involves formation of the 5′-hydrazo substituted compounds, which are most likely formed due to the rearrangement of an intermediate nucleoside-PPh_3/DEAD complex.[85] Moreover, the

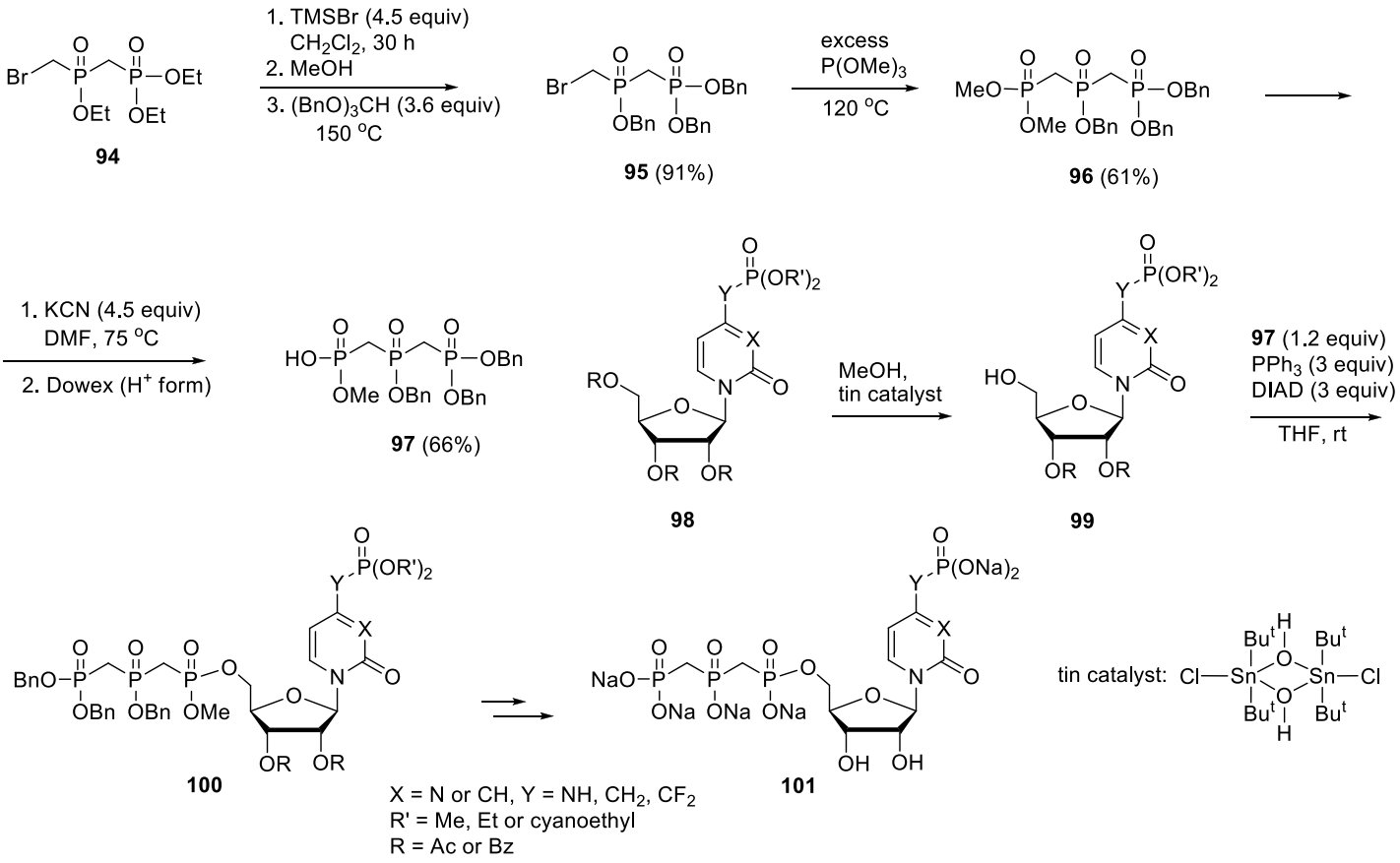
Mitsunobu reaction is a poor choice if an incoming phosphonate substitute has several hydroxyl groups available for coupling.[86,87] Nevertheless, in spite of these limitations, several recent innovations have significantly extended the scope and synthetic utility of the method.[86,88,89] Thus, Taylor and co-workers have developed an unsymmetrical approach to the synthesis of bismethylene triphosphate analogue **86** via sequential Michaelis-Arbuzov reactions on bis-halomethylenephosphinates. The ester **86** was mono-deprotected at one of the terminal phosphonate groups by reaction with 1.0 equiv of KCN in DMF at 70 °C.
The resulting monodeprotected compounds **87** were used to achieve the first synthesis of the bismethylene analogues of UTP and CTP. Acid **87a** was coupled to 2′,3′-*O*,N^3-tribenzoyluridine **88** via the Mitsunobu reaction to give **90**. However, while this reaction smoothly proceeded to give 79% yield of the product, the authors had to use the triethylammonium salt **87b** to get a good yield in the case of 2′,3′-*O*,N^3-tribenzoylcytidine **89**. Complete deprotection of **90** and **91** was achieved by subjecting them to bromotrimethylsilane followed by treatment with aq. NH_4OH-MeOH (Scheme 36).[86]



Scheme 36. Synthesis of the bismethylene analogues of UTP and CTP.

Another example of successful application of the Mitsunobu coupling is the synthesis of bismethylene triphosphate nucleotides of uridine 4-phosphate analogues **101**. Bismethylene triphosphate derivative **97**, a phosphorus component in the synthesis of **101**, was prepared by a Michaelis-Arbuzov route from compound **94**. The selective cleavage of 5′ ester moiety of 2′,3′,5′-tri-*O*-acetyl or tri-*O*-benzoyl U-4-P analogues **98** was accomplished with the aid of a tin catalyst. The Mitsunobu coupling of 5′-deprotected U-4-P analogues **99** to an unsymmetrical bismethylene triphosphate bearing a free phosphonic acid moiety at one of the terminal

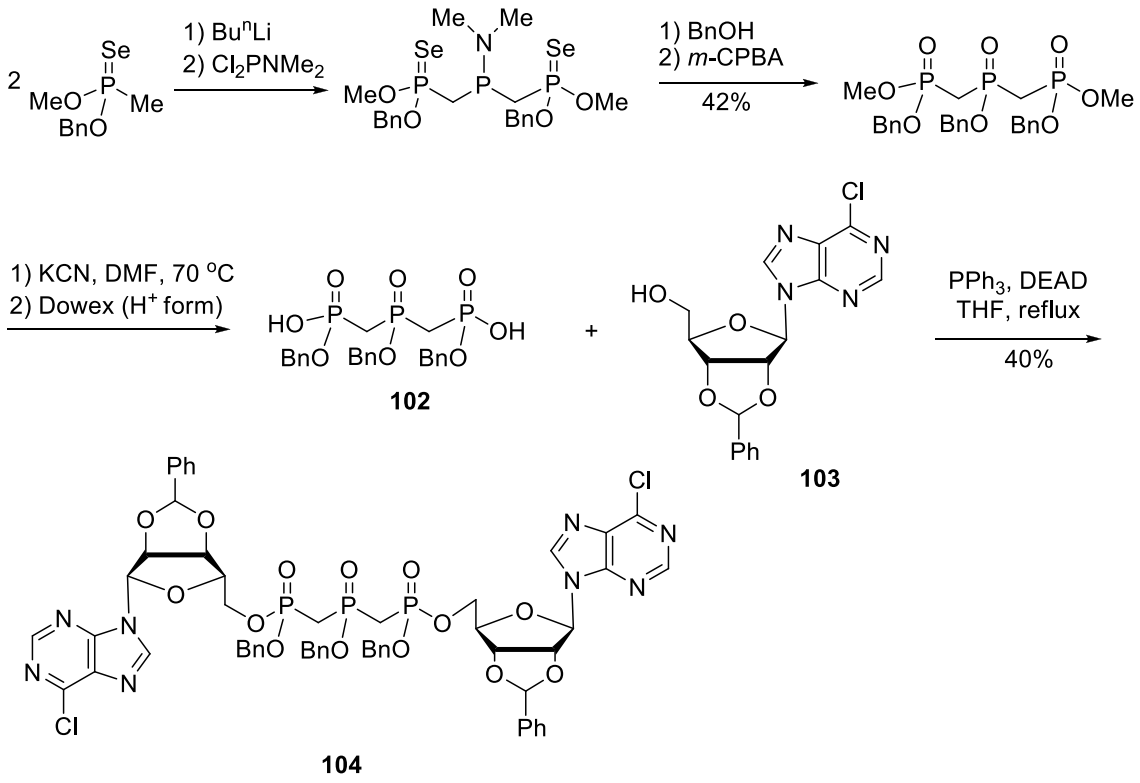
positions gave fully protected bismethylene triphosphate U-4-P analogues **100**. Global deprotection of nucleotides **100** was carried out by treatment with 6-9 equiv of TMSBr followed by ammonium hydroxide in methanol (Scheme 37).[87]



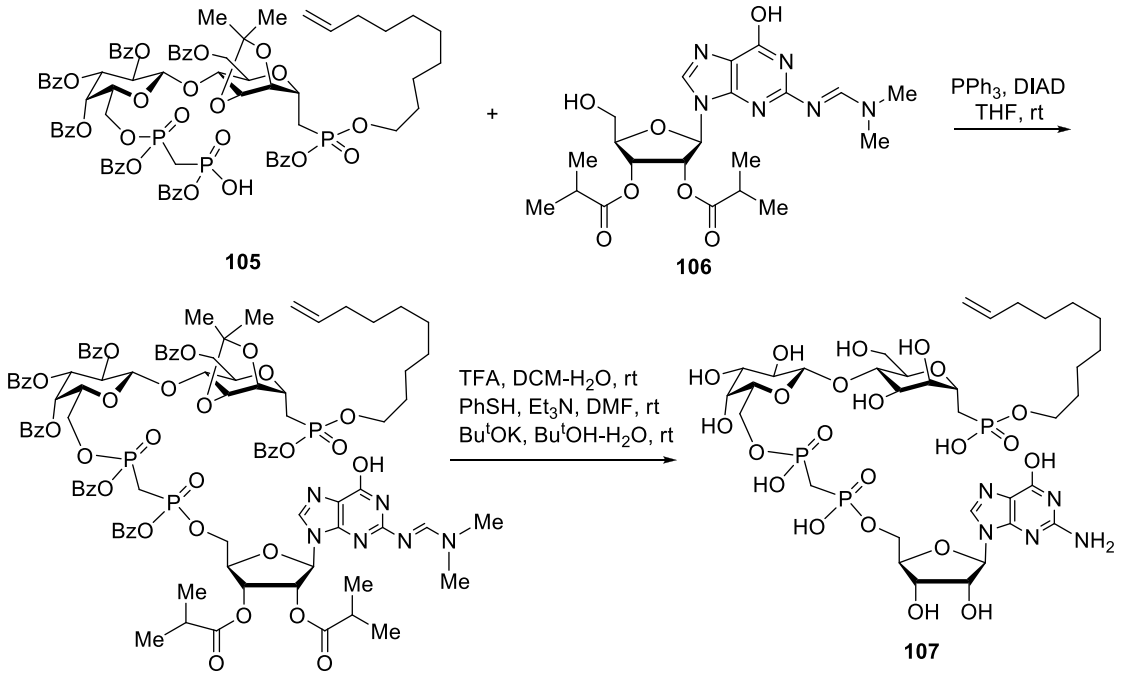
Scheme 37. Synthesis of bismethylene triphosphate nucleotides of uridine 4-phosphate analogues.

To prepare enzymatically and chemically non-hydrolyzable analogues of dinucleoside triphosphates Ap_3A and Gp_3G, Lebeau and co-workers have developed a new methodology based on *O*,*O*-dialkyl seleno-phosphonate chemistry.[85,90] The bisphosphonic acid **102**, a key building block in the synthesis of dinucleoside triphosphate analogues $ApCH_2pCH_2pA$ and $GpCH_2pCH_2pG$, was prepared via a one-pot condensation / transesterification / oxidation / dealkylation sequence involving *O*,*O*-dialkyl methaneselenophosphonates. The bisphosphonic acid was then condensed with 2′,3′-*O*-benzylidene-6-chloroadenosine **103** under modified conditions of the Mitsunobu reaction to afford dinucleoside triphosphate analogue **104** in 40% yield (Scheme 38). The diguanosine derivative was prepared using a similar strategy.

The Mitsunobu esterification was found to be particularly effective for the preparation of potential bisubstrate inhibitors of *Leishmania* elongating α-D-mannosyl phosphate transferase.[91] Thus, coupling between the phosphonodisaccharide methylenebisphosphonate derivative **105** and the guanosine derivative **106** is a crucial step of the synthesis of the required transition state analogue **107** in which a guanosine moiety is linked to the acceptor substrate through the methylenebisphosphonate bridge, mimicking the important guanosine-pyrophosphate motif present in the natural substrate donor GDP-mannose (Scheme 39).



Scheme 38. Synthesis of dinucleoside triphosphate analogue $ApCH_2pCH_2pA$.

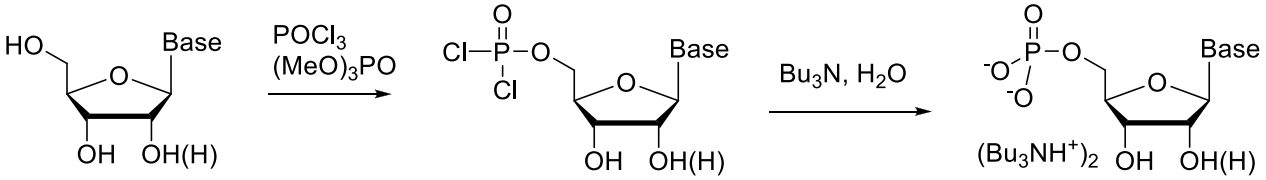


Scheme 39. Mitsunobu esterification in synthesis of a potential mechanism-based bisubstrate inhibitor **107**.

2.4. Electrophilic phosphorylation of nucleosides by the Yoshikawa and Ludwig-Eckstein approaches

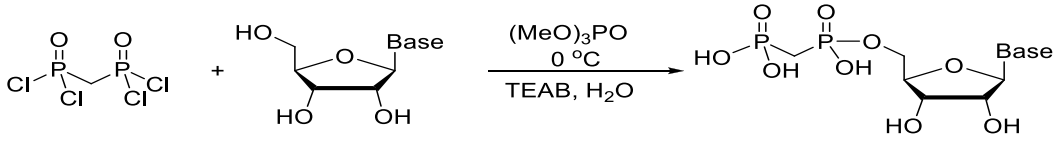
The Yoshikawa procedure involves the selective 5´-monophosphorylation of a nucleoside with the electrophilic phosphorus oxychloride ($POCl_3$) using trimethyl or triethyl phosphate as the solvent (Scheme 40).[92]

Yoshikawa's initial studies were performed on 2′,3′-*O*-isopropylidene-protected NTPs, but later it was found that selective reaction at the 5′-OH was possible for unprotected NTPs and dNTPs. An acidic medium was reported to be critical for selective reaction at the 5′-hydroxyl for unprotected NTPs and dNTPs. In particular, the addition of water to the phosphorylating reagent results in selective 5′-phosphorylation of nucleosides in moderate to high yield.[93] Nevertheless, the literature data on the phosphorylation with $POCl_3$ are contradictory and reveal that good regioselectivity can be also obtained when the medium is slightly basic, so the relationship between regioselectivity of phosphorylation and pH remains unclear.[55] Yoshikawa and co-workers also used pyrophosphoryl chloride in place of $POCl_3$ but reported no significant advantages.[93] Thiophosphoryl derivatives of nucleotides can be also obtained via a Yoshikawa procedure that employs $PSCl_3$ to generate 1-thiotriphosphates.[94]



Scheme 40. Yoshikawa approach for the synthesis of nucleoside monophosphates.

In 2005, Darzynkiewicz and co-workers developed a method for direct phosphonylation of unprotected nucleosides with methylenebis(phosphonic dichloride) using Yoshikawa's conditions.[95] They found that $CH_2(POCl_2)_2$ is more reactive than $POCl_3$ and reactions with this reagent proceeded faster than those using $POCl_3$. The products of diphosphonylation, nucleoside 5′,3′- or 5′,2′-*O*-di-[methylenebis(phosphonate)]s, were observed only after long periods of time or when a very high (4–10 equiv) excess of $CH_2(POCl_2)_2$ was used. The reactions proceeded in mild conditions and afforded the nucleoside 5′-methylenebis(phosphonate)s in high yields. This method was successfully applied in the synthesis of dinucleoside polyphosphates and trimethylguanosine cap analogues modified in the triphosphate bridge[47,49,50,96-100] (Scheme 41).

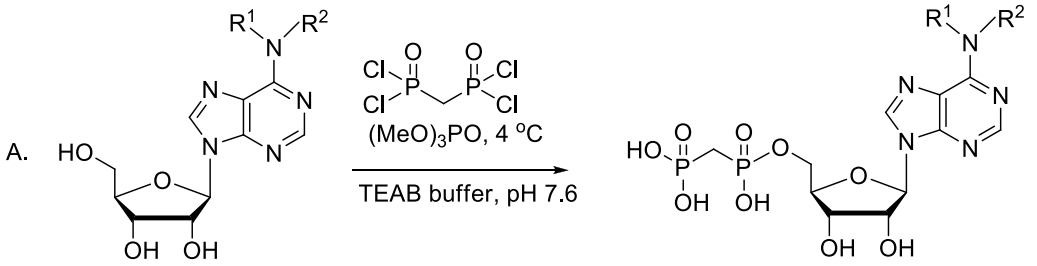


Nucleoside	Equivalents of $CH_2(POCl_2)_2$	Reaction time	HPLC yield (%)
Guanosine	2	1 h	85
2',3'-*O*-Isopropylidene guanosine	2	1h	89
3'-*O*-Methyl guanosine	2	1h	81
7-Methyl guanosine	4	45 min	81
Adenosine	2	4 h	77
Inosine	2	35 min	75
Uridine	6	9 h	65
Cytidine	2	1h	89

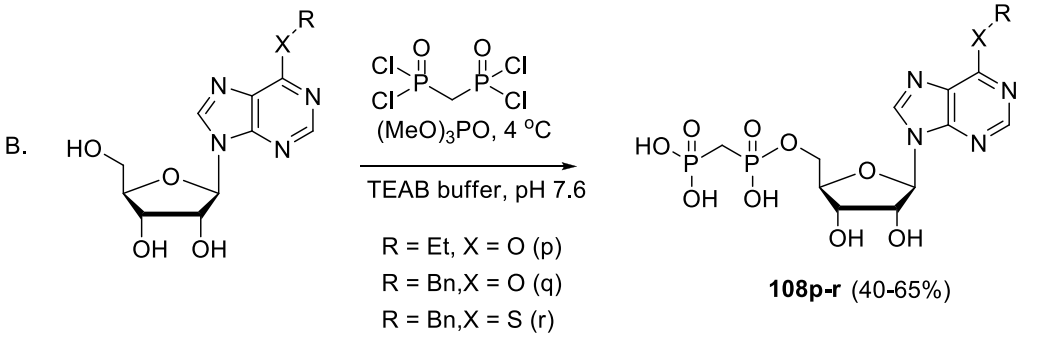
TEAB: triethylammonium bicarbonate

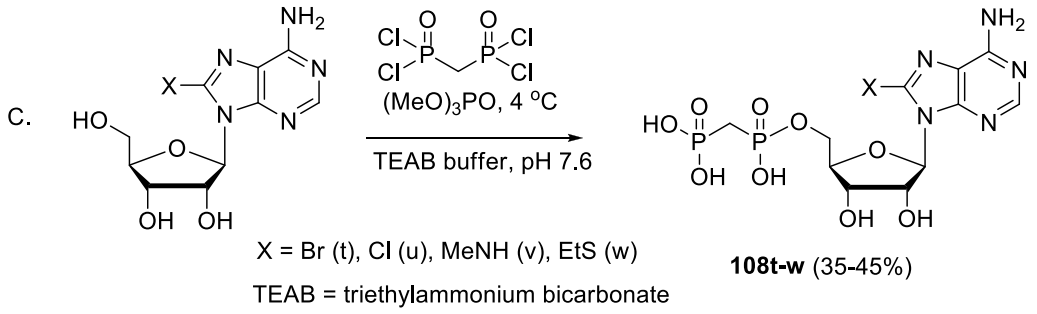
Scheme 41. Electrophilic phosphorylation of nucleosides by the Yoshikawa approach.

Several examples (A-C) of "one-pot, two-step" preparation of functionalized α,β-CH_2-ADP derivatives **108** via Yoshikawa procedure are shown in Scheme 42.[32] These *ecto*-5′-nucleotidase inhibitors have potential application as novel therapeutics for melanomas, lung, prostate and breast cancers. The most potent inhibitors were N^6-(4-chlorobenzyl)- (**108l**, K_i 7.23 nM), N^6-phenylethyl- (**108h**, K_i 8.04 nM), and N^6-benzyl-adenosine (**108g**, K_i 9.03 nM) derivatives. Replacement of the 6-NH group in **108g** by O (**108q**) or S (**108r**) yielded equally potent inhibitors.



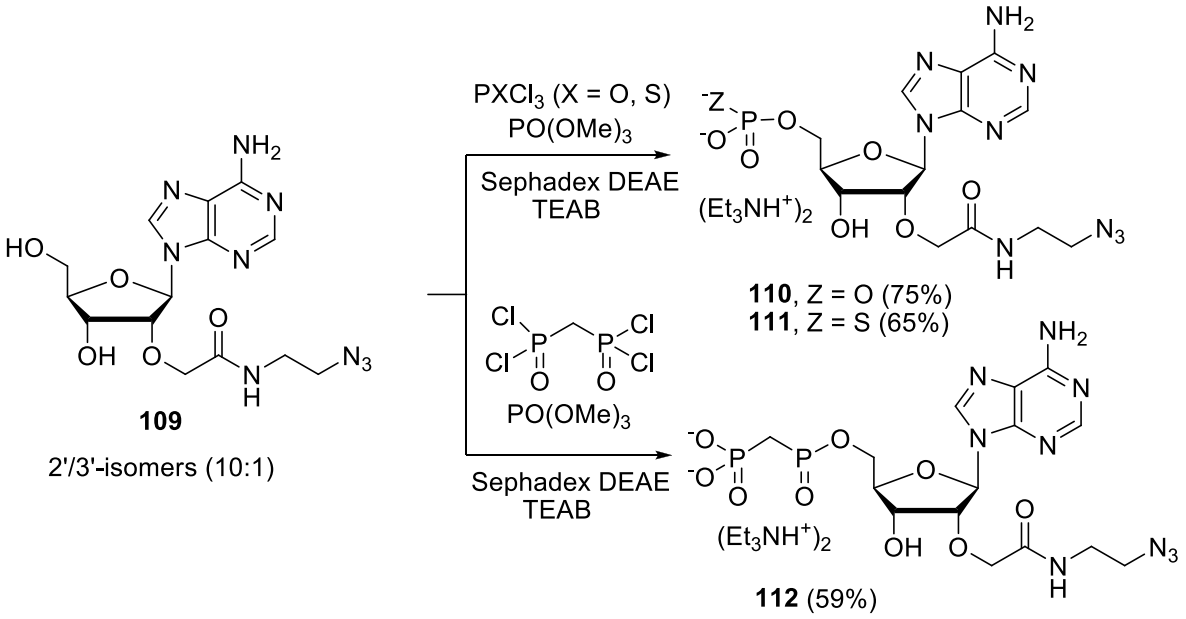
compd	R^1	R^2	compd	R^1	R^2
a	Me	H	h	2-phenylethyl	H
b	Et	H	i	Bn	Et
c	Me	Me	j	Bn	Bn
d	Et	Et	k	4-aminobenzyl	H
e	Et	Me	l	4-chlorobenzyl	H
f	Ph	H	m	4-nitrobenzyl	H
g	Bn	H	n	4-sulfamoylbenzyl	H





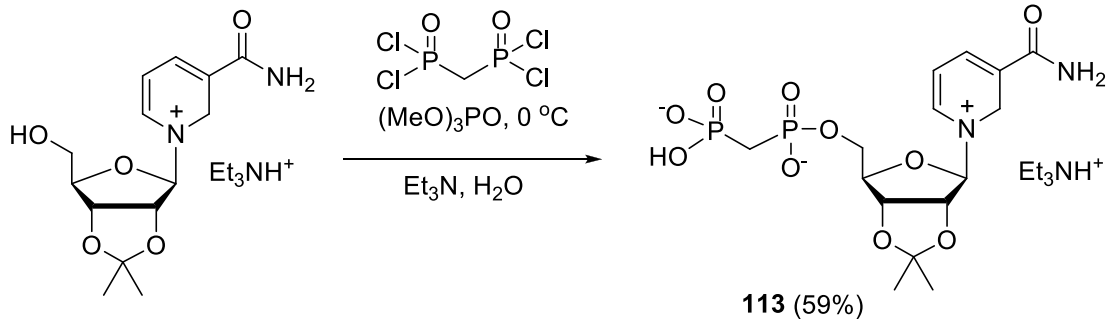
Scheme 42. Synthesis of *ecto*-5′-nucleotidase inhibitors via the Yoshikawa procedure.

Modified Yoshikawa phosphorylation procedures were employed to convert nucleoside **109** bearing a "clickable" azido linker at 2′-position of adenosine into its 5′-monophosphate **110** or 5′-monophosphorothioate **111** using $POCl_3$ or $PSCl_3$. Similarly, CH_2-modified ADP **112** was obtain by reacting **109** with methylenebis(phosphonic dichloride) (Scheme 43).[101]



Scheme 43. Synthesis of 5′-phosphate- and 5′-methylenebisphosphonate derivatives of 2′-*O*-[*N*-(2-azidoethyl)-carbamoyl]methyladenosine.

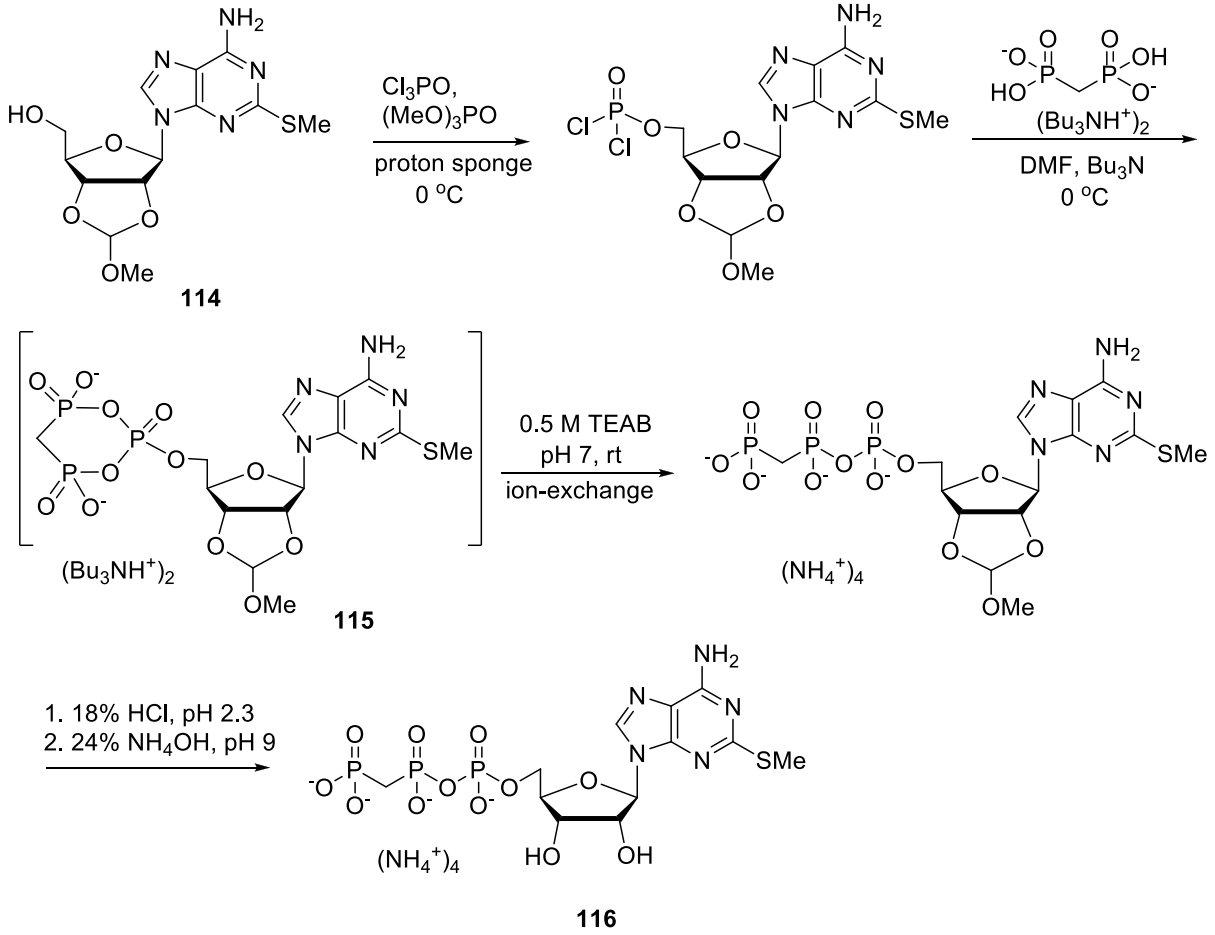
The Yoshikawa approach was employed for the synthesis of the zwitterionic nicotinamide ribosyl methylenebisphoaphonate **113**. Treatment of nicotinamide-β-D-ribose 2′,3′-isopropylidene acetal with a 3-fold excess of $CH_2(POCl_2)_2$ in $PO(OMe)_3$ followed by adding the mixture to aqueous triethylamine affords compound **113** in 59% yield (Scheme 44).[102]



Scheme 44. Synthesis of nicotinamide riboside 5′-methylenebis(phosphonate) 2′,3′-*O*-isopropylidene acetal mono(triethylammonium) salt.

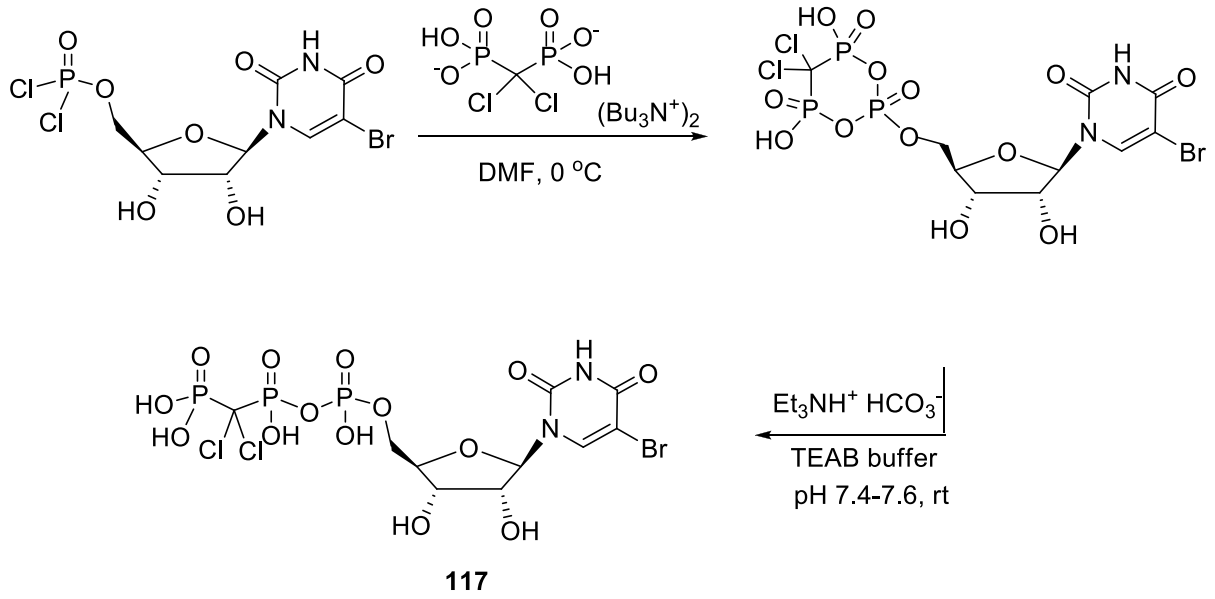
An important feature of Yoshikawa monophosphorylation reactions is that phosphorodichloridate intermediates can be used directly for the synthesis of nucleoside triphosphates. Ludwig[103] and Ruth[94] have shown that treatment of phosphorochloridates, generated in situ via Yoshikawa procedure, with bis(tri-*n*-butylammonium) pyrophosphate in dry DMF affords the nucleoside triphosphates in good yields. This

approach was successfully adopted for the synthesis of β,γ-methylene-substituted nucleoside triphosphates. Thus, Fisher and co-workers have proposed a short one-pot synthesis of 2-MeS-β,γ-CH_2-ATP (**116**) represented in Scheme 45.[104] To ensure a selective reaction of 2-methylthioadenosine at 5′-OH, they used 2′,3′-methoxymethylidene-2-methylthioadenosine **114** as the starting material. Nucleoside **114** was first treated with Cl_3PO in $(MeO)_3PO$ in the presence of 1,8-bis(dimethylamino)naphthalene (proton sponge), followed by the addition of bis(tributylammonium)methylenebisphosphonate and tributylamine. Finally, hydrolysis of the cyclic intermediate **115** and deprotection of the methoxymethylidene group afforded **116** in 35% overall yield.



Scheme 45. Application of Yoshikawa approach to the synthesis of a β,γ-CH_2 ATP analogue.

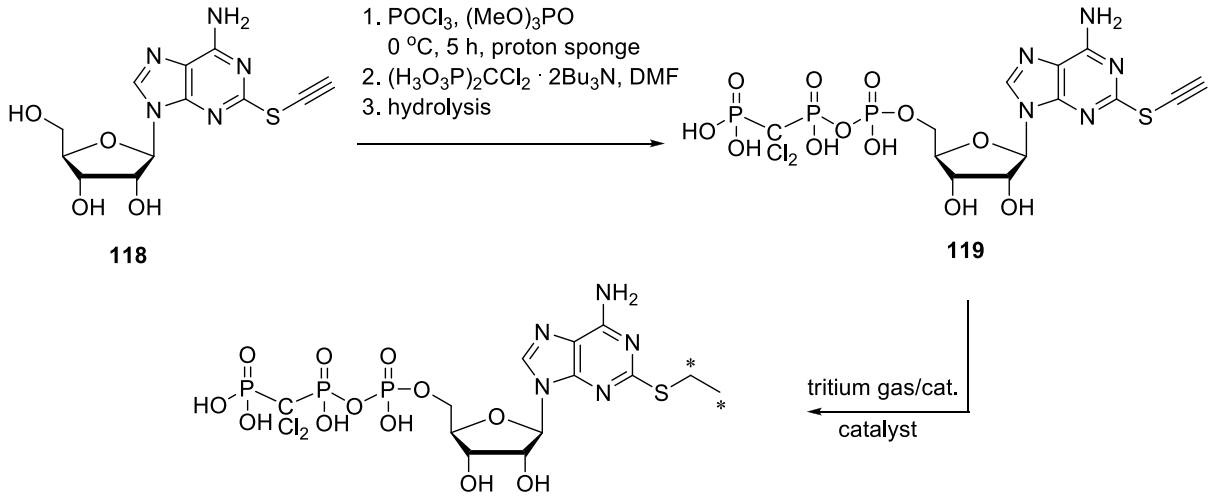
The fact that P2Y receptors have been found to be implicated in a variety of pathophysiological states such as vascular, inflammatory, and immune diseases pushed Müller's team to synthesize a series of UTP, UDP, and UMP derivatives and analogues modified in the uracil part of the molecule.[105] Thus, a triphosphate-analogous structure containing a β,γ-dichloromethylene bridge was successfully introduced into 5-bromouridine via Yoshikawa approach, yielding the nucleotide analogue **117** as shown in Scheme 46. A β,γ-dichloromethylene modification in the triphosphate chain of 5-bromo-UTP was tolerated by all three receptor subtypes, thus opening up a new strategy to obtain ectonucleotide diphosphohydrolase- and phosphatase-resistant $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptor agonist.



117

Scheme 46. Synthesis of β,γ-dichloromethylene-substituted 5-bromo-UTP analogue.

A similar procedure was used by Müller and co-workers for the preparation of new β,γ-CCl_2 substituted ATP based ^{3}H-labeled radioligand **120** ([^{3}H]PSB-0413) (Scheme 47).[106] As a precursor for tritiation authors selected the corresponding propargyl derivative. Reaction of the nucleoside **118** with phosphorus oxychloride in trimethyl phosphate followed by reaction with dichloromethylenediphosphonic acid in DMF afforded the corresponding triphosphate analogues **119**. The latter was subsequently subjected to catalytic hydrogenation using tritium gas. In preliminary saturation binding studies, [^{3}H]PSB-0413 showed high affinity for platelet $P2Y_{12}$ receptors with a K_D value of 4.57 nM.



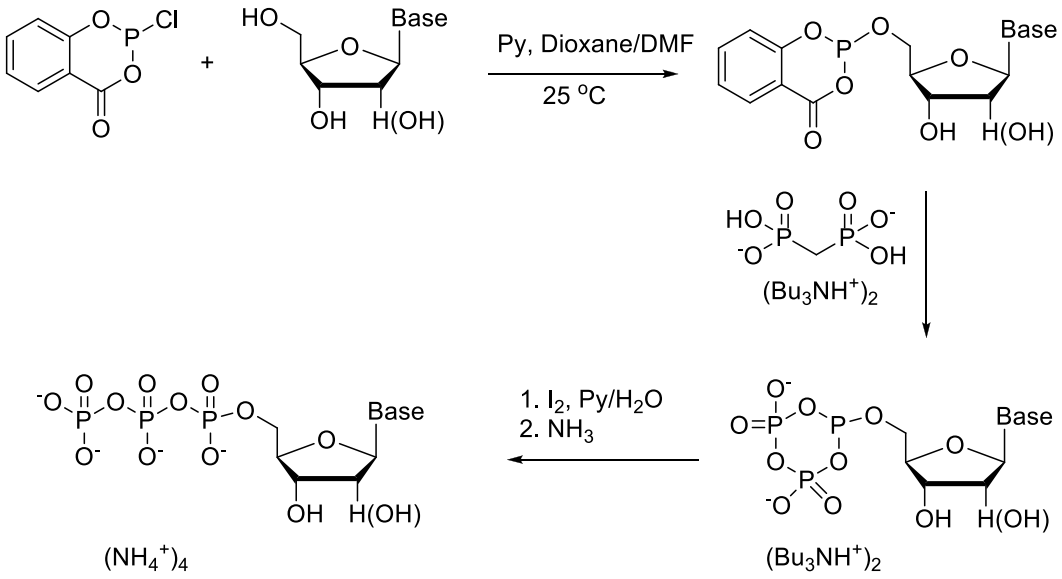
118 **119**

120 (PSB-0413)

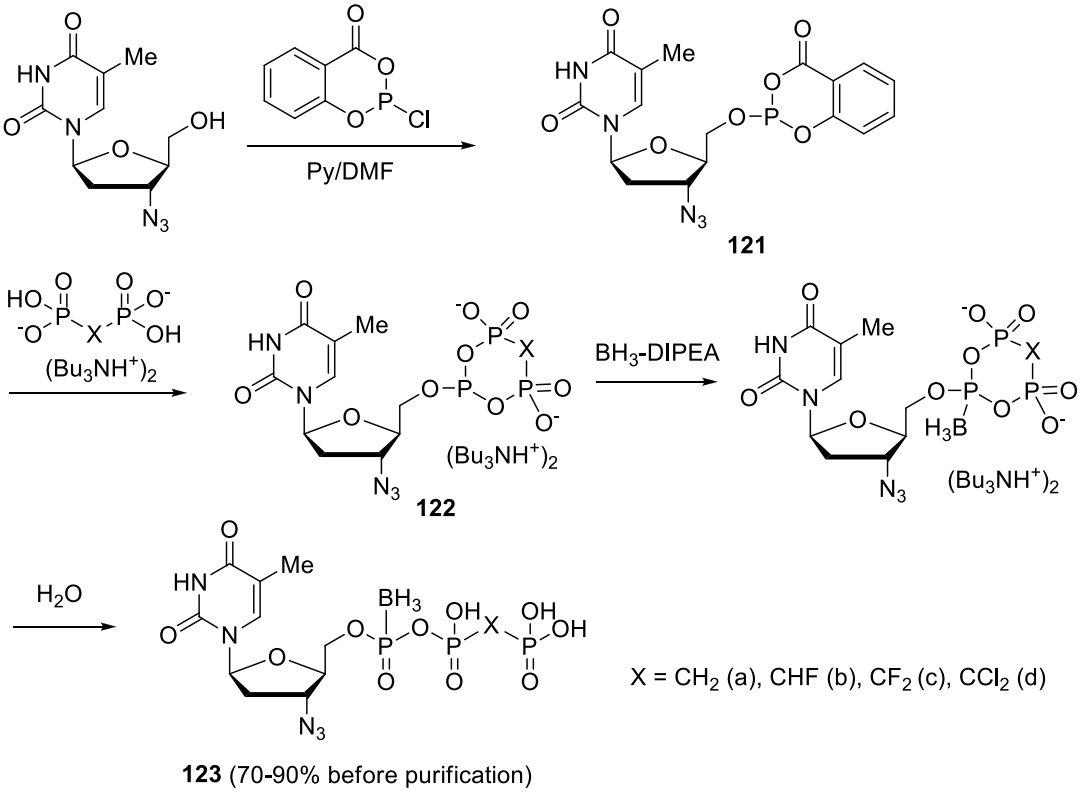
Scheme 47. Synthesis of nucleotide analogue [^{3}H]PSB-0413 via the Yoshikawa procedure.

In 1989, Ludwig and Eckstein published a modification of electrophilic phosphorylation that employs 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one.[107] This reaction gave an activated phosphite that was reacted

with pyrophosphate to form the cyclic intermediate. The latter can be oxidized and hydrolyzed to give the corresponding triphosphate (Scheme 48). It was shown that protection of nucleobase functionality for A, T, G, and C was not required, but selectivity for the 5´-hydroxyl in the initial phosphitylation step was marginal if the 3´- and the 2´-hydroxyl were not protected.[55]



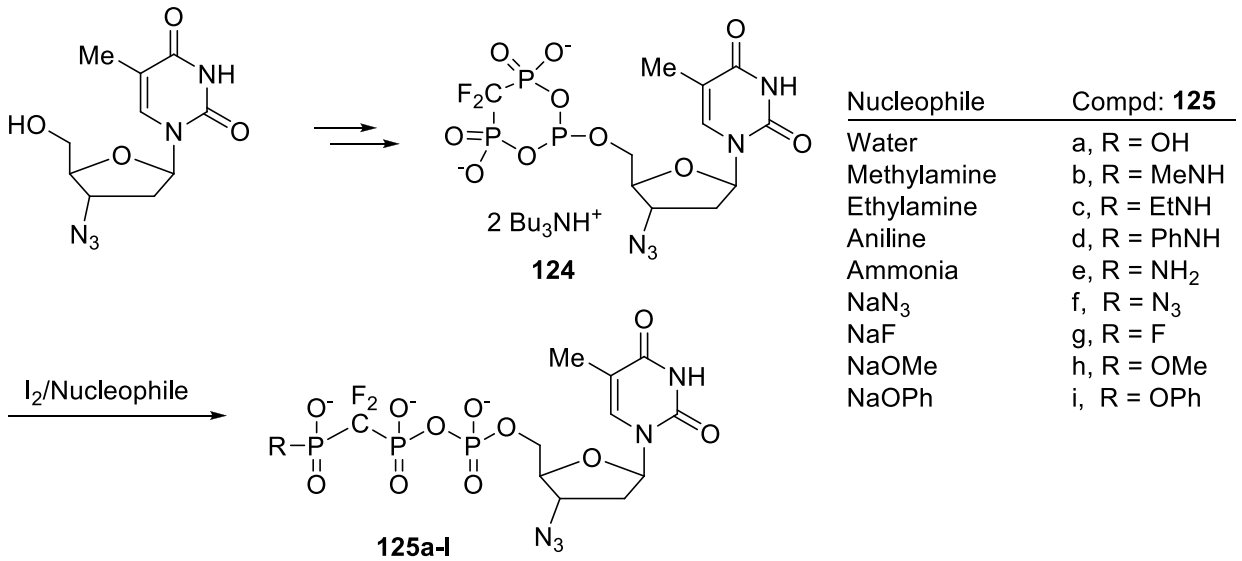
Scheme 48. Ludwig-Eckstein electrophilic phosphorylation of nucleosides.



Scheme 49. Synthesis of AZT 5´-α-*P*-borano-β,γ-bridge-modified triphosphates.

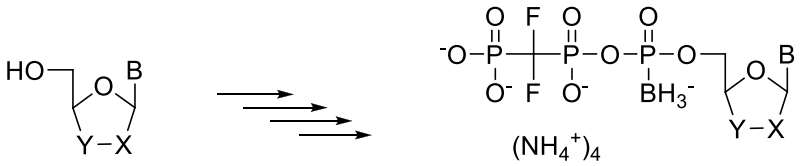
The usefulness of the Ludwig-Eckstein approach in the development of a convenient synthetic route to β,γ-methylene modified nucleotides was demonstrated by Wang and co-workers who reported the synthesis of AZT 5′-triphosphate mimics **123**.[108] Thus, reaction of AZT with 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, followed by treatment of the phosphite intermediate **121** with a bisphosphonate salt, yielded the cyclic triphosphate analogues **122**, which were subjected to boronation and subsequent hydrolysis to give AZT 5′-α-*P*-borano-β,γ-bridge-modified triphosphates **123** in moderate to good yields (Scheme 49).

Interestingly, α-*P*-BH_3^- β,γ-CF_2 AZTTP analogue **123c** was equipotent in inhibition of HIV-1 RT to AZTTP and more potent than its β,γ-*O* analogue. Reaction of the cyclic intermediate **124** with iodine, followed by treatment with a series of nucleophiles, afforded the AZT 5′-β,γ-difluoromethylene-γ-substituted triphosphates (**125b**-**l**) (Scheme 50).



Scheme 50. Synthesis of AZT 5′-γ-*P*-substituted β,γ-(difluoromethylene)-triphosphates.

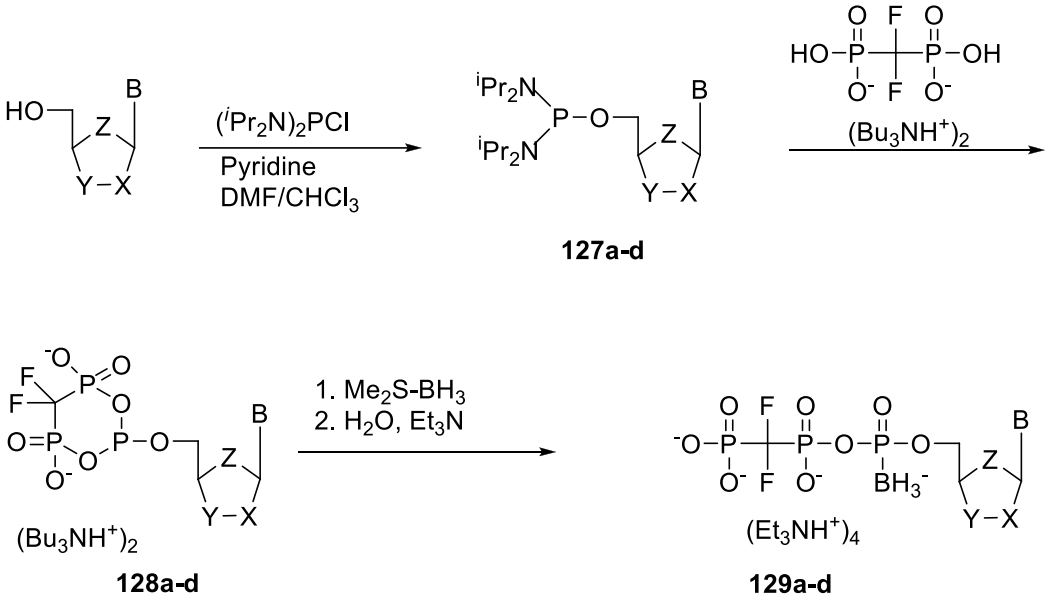
Synthesis of a series of 2′,3′-dideoxynucleoside 5′-α-*P*-borano-β,γ-(difluoromethylene)-triphosphates, ddN5′-αB-β,γ-CF_2 TPs, and their inhibitory properties on HIV-1 RT have also been studied (Scheme 51).[109] Compounds **126** were prepared according to a similar procedure for preparation of AZT 5′-αB-β,γ-CF_2 TPs (see Scheme 49). However this synthetic route did not apply well to the nucleosides having an exocyclic amino group; therefore, an alternative synthetic procedure was developed (Scheme 52). The course of the reactions was similar to that in Scheme 49 except that the bis(diisopropylamino)phosphites were the active phosphite intermediates. Treatment of **127** with bis(tributylammonium) difluoromethylenediphosphonate presumably yielded the cyclic intermediates **128**, which were subsequently subjected to boronation and hydrolysis to give **129**. All the resulting ddN5′-αB-β,γ-CF_2 TPs demonstrated essentially the same level of inhibition of HIV-1 RT as the corresponding ddNTPs. Given their enhanced biological stability, these compounds represent a new class of potential antiviral agents.[109]



126a-f

a: B = thymine, X = Y = CH_2; **b**: B = thymine, X, Y = CH=CH;
c: B = 5-F-cytosine,X = CH_2, Y = S (L-ribose); **d**: B = uracil, X = Y = CH_2;
e: B = adenine, X = Y = CH_2; **f**: B = 7-deazaguanine, X = Y = CH_2

Scheme 51. Synthesis of the triphosphate mimics of antiviral 2′,3′-dideoxynucleosides.



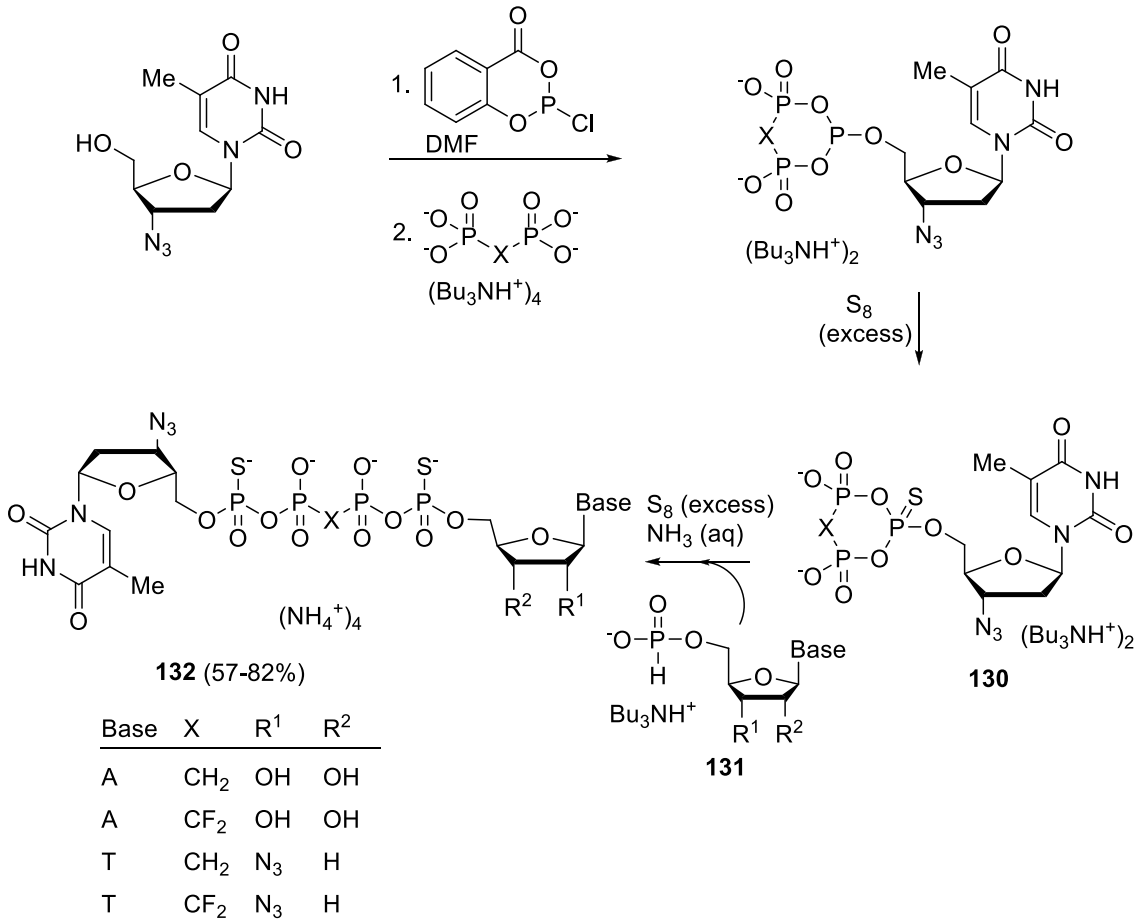
127a-d

128a-d **129a-d**

a: B = cytosine, X = Y = CH_2, Z = O; **b**: B = cytosine, X = CH_2, Y = S (L-ribose), Z = O;
c: B = thymine, X = CH_2, Y = CHF (F on α-face), Z = O; **d**: B = guanine, X = Y = CH, Z = CH_2

Scheme 52. Synthetic pathway for the preparation of ddN 5′-αB-β,γCF_2 TPs.

The modified Ludwig-Eckstein protocol with methylenebisphosphonic acids was also successfully employed for the preparation of AZT tetraphosphate mimics **132** as depicted in Scheme 53. Oxidation of phosphite intermediates with sulfur followed by condensation of **130** with H-phosphonate monoesters **131** (in the presence of excess S_8) opens a route to nucleotide analogues AZTp$_S$p$_{CX2}$pp$_S$A containing two outer thiophosphate moieties and a central bisphosphonate, and related compounds AZTp$_S$p$_{CX2}$pp$_S$AZT with AZT at both ends. This family of compounds is a hydrolysis-resistant version of the AZTppppA that results from excision of AZT by AZT- resistant HIV reverse transcriptase and therefore may be useful in drug design.[110]

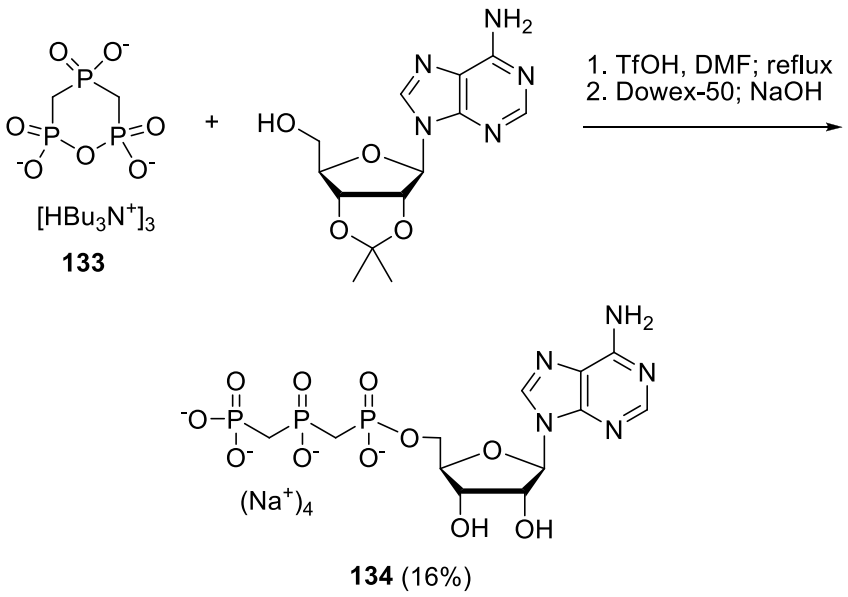


Base	X	R^1	R^2
A	CH_2	OH	OH
A	CF_2	OH	OH
T	CH_2	N_3	H
T	CF_2	N_3	H

Scheme 53. Synthesis of AZT tetraphosphate mimics via the modified Ludwig-Eckstein procedure.

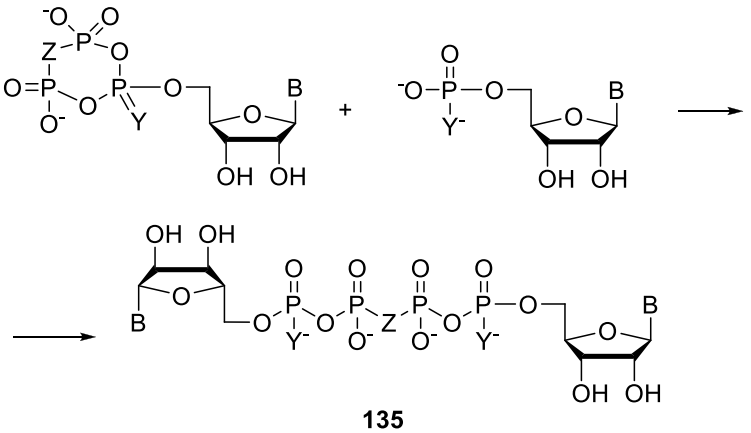
2.5. Synthesis involving nucleophilic cleavage of cyclic trimetaphosph(on)ates

Kenyon *et al*. reported the synthesis of the anhydride of bismethylenetriphosphonic acid **133** via DCC-mediated condensation of bismethylenetriphosphonic acid, $HO(O)P[CH_2P(O)(OH)_2]_2$.[111] The same group has described ring-opening of **133** by 2′,3′-isopropylideneadenosine in a polar aprotic solvent at an elevated temperature in the presence of a strong acid. This led to a simple synthesis of α,β;β,γ-bismethylene analogue of ATP **134** as shown in Scheme 54.[112] Attempts to use this approach for the synthesis of phosphonate analogue of thymidine triphosphate were unsuccessful.[25]

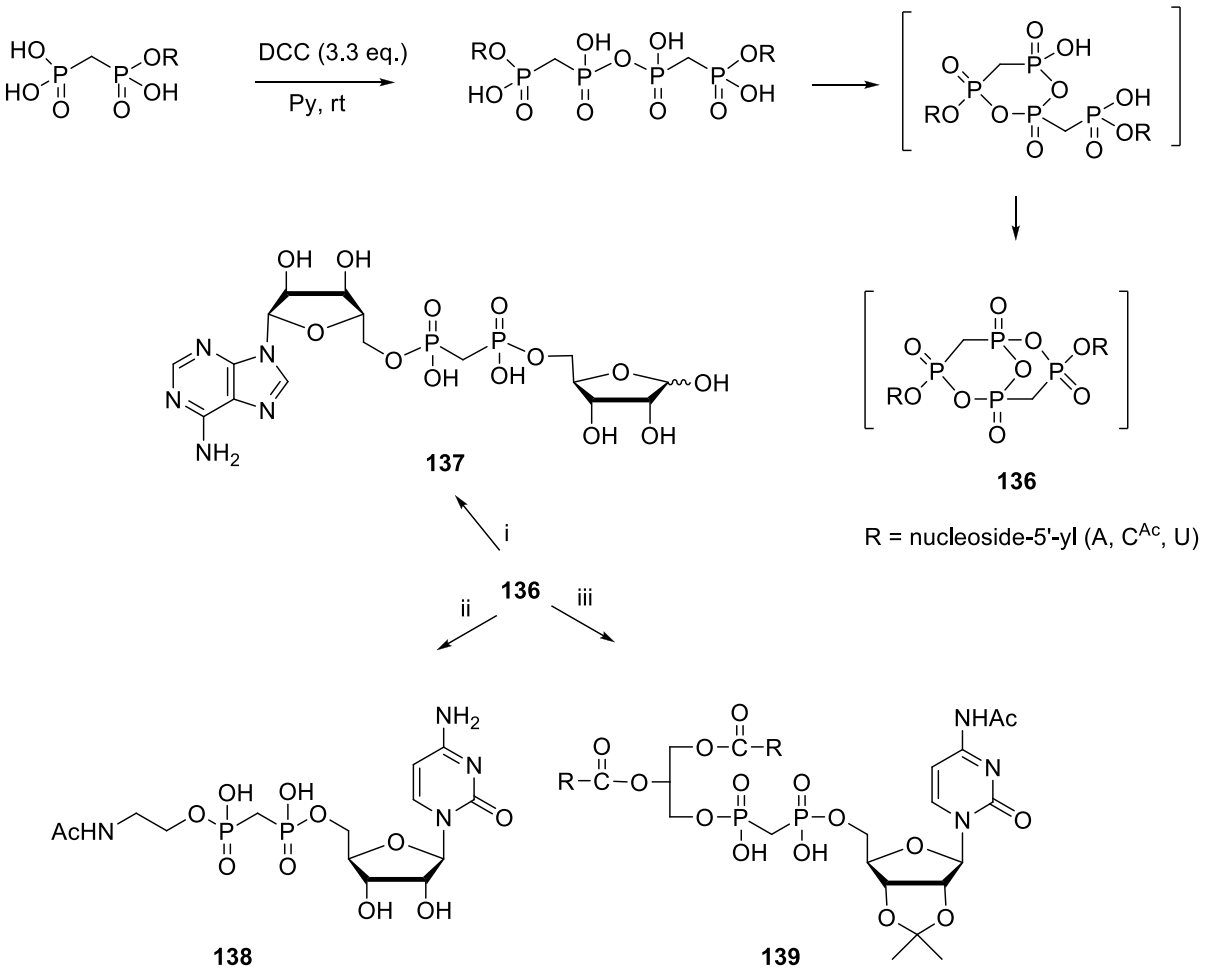


Scheme 54. Synthesis of α,β;β,γ-bis-CH_2 ATP via anhydride of bismethylenetriphosphonic acid.

Another approach to methylene-modified nucleoside polyphosphates via ring-opening reactions is based on the reactions of a P^1,P^3-cyclic nucleoside trimetaphosph(on)ates which can be prepared by treatment of the corresponding nucleoside triphosph(on)ate analogues with carbodiimides or phosphitylation of the 5′-hydroxy group of 2′,3′-protected nucleosides, followed by double substitution of salicylate with bis-phosphonates and oxidation of the resulting cyclic phosphite (see also Section iv). For example, an effective method for the synthesis of bis-nucleoside tetraphosphate analogues **135** involves the treatment of nucleoside trimetaphosph(on)ates with nucleoside monophosphates or monothiophosphates (Scheme 55).[77]



Scheme 55. Synthesis of bis-nucleoside tetraphosphate analogues from nucleoside P^1,P^3-cyclic triphosph(on)ates.



Reagents and conditions: (i) benzyl 2,3-*O*-isopropylidene-β-D-ribose; (ii) *N*-acetylethanolamine; (iii) 1,2-dipalmitoyl-*sn*-glycerol. Heating in pyridine at 65 °C, then hydrolysis (H_2O, rt).

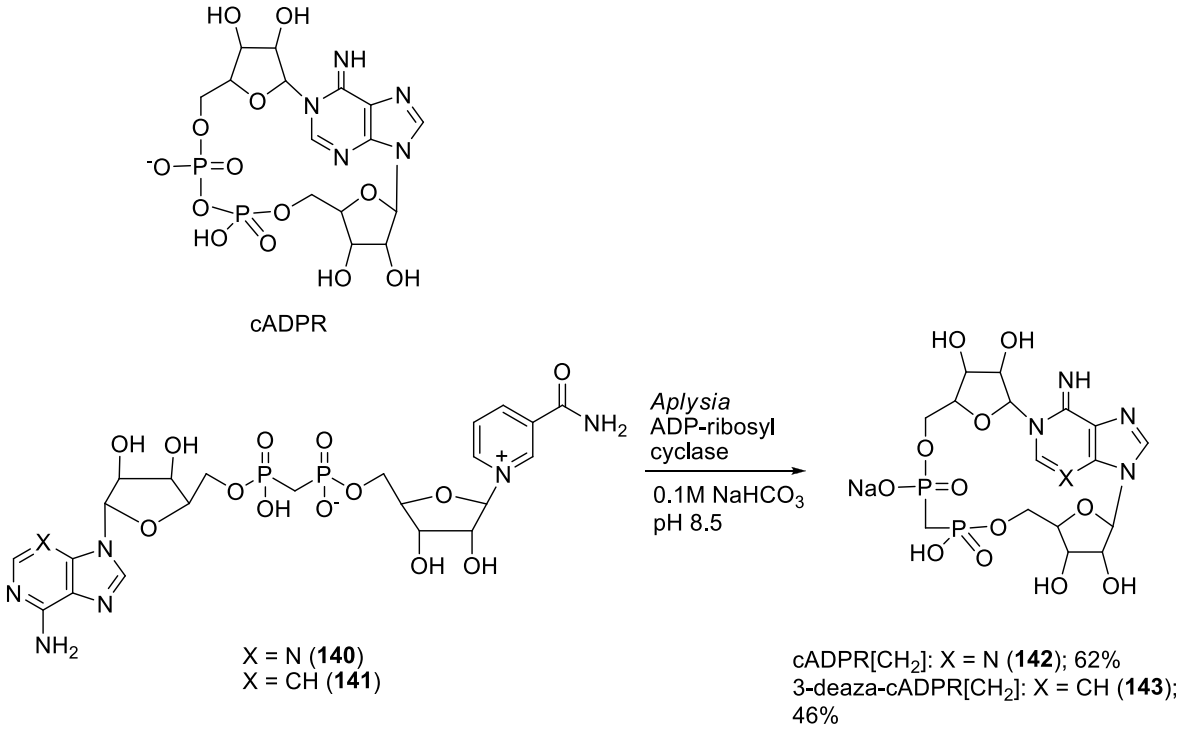
Scheme 56. Synthesis of methylenebisphosphonate analogues of P^1,P^2-disubstituted pyrophosphates via bicyclic trisanhydrides.

Pankiewicz and co-workers have developed a synthesis of the novel nucleoside bicyclic trisanhydrides **136** in the reaction of nucleoside-5´-methylenebisphosphonates with DCC. These authors took advantage of generated anhydrides as intermediates in the synthesis of methylenebisphosphonate analogues of P^1,P^2-disubstituted pyrophosphates. Thus, the reaction of **136** with benzyl 2´,3´-*O*-isopropylidene-β-D-ribose followed by hydrolysis and deprotection afforded ADP-ribose analogue **137** in 72% overall yield. Treatment of **136** (R = C^{Ac}) with *N*-acetylethanolamine or 1,2-dipalmitoyl-*sn*-glycerol gave methylenebisphosphonate analogues of CDP-ethanolamine and CDP-DAG (**138** and **139**, respectively), in high yield (Scheme 56).[113]

2.6. Enzyme-mediated reactions

Several review articles highlight developments in this field.[17,55,114] Enzymatic phosphorylation was shown to be an ideal method for certain applications. However, enzyme-mediated reactions do not allow their routine use for the synthesis of nucleotides with unnatural base, sugar and polyphosphate residues. Worth quoting are Burgess and Cook who noted that "enzyme-mediated syntheses of unnatural nucleoside triphosphates are only cost-effective if the expected advantages of this approach are likely to offset the costs of the additional

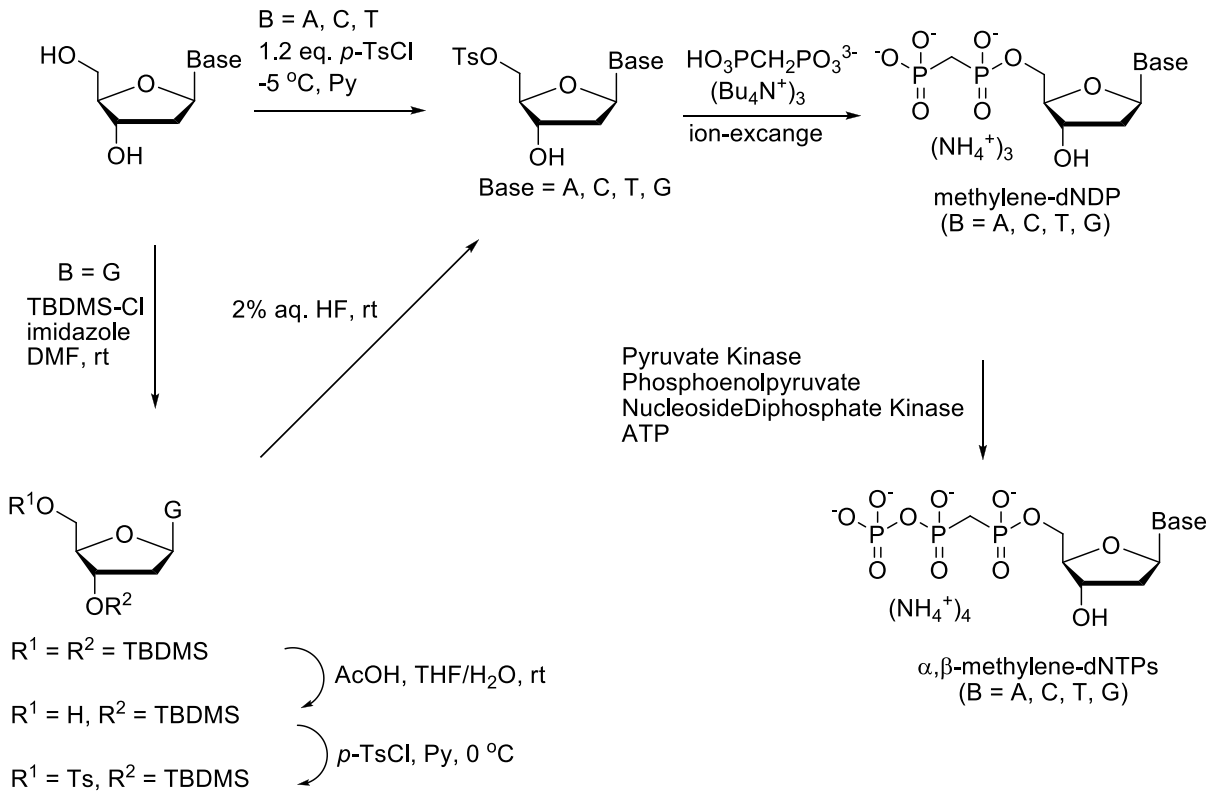
development time required".[55] The merits of enzymatic methods are their minimal need for protection / deprotection steps and the regio- and stereo-chemical unambiguity of biocatalytic reactions. A combination of chemical and enzymatic methods has particular utility in cases where the reactivity of the nucleobase precludes the use of electrophilic phosphorylation reagents. An elegant example of this technique was reported by Slama and co-workers who carried out the synthesis of the two novel cyclic ADP-ribose (cADPR) analogues **142** and **143** via cyclization of the corresponding linear nicotinamide adenine dinucleotides (NADs) **140** and **141** catalyzed by *Aplysia californica* ADP-ribosyl cyclase (Scheme 57).[102]



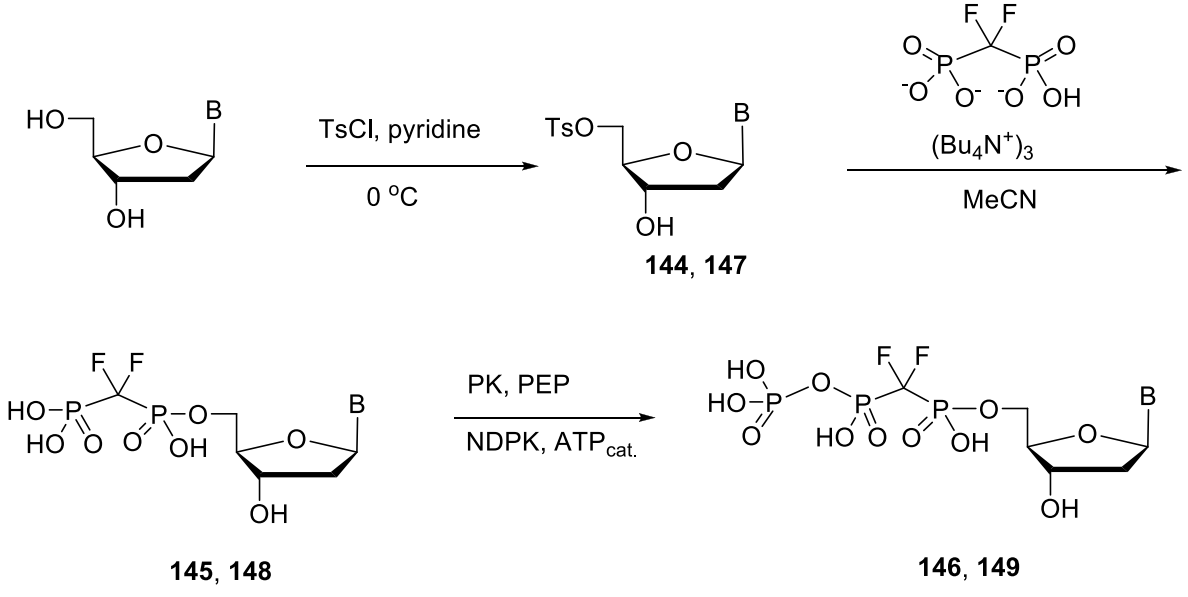
Scheme 57. *Aplysia* ADP-ribosyl cyclase-catalyzed synthesis of cADPR[CH_2] (**142**) and 3-deaza-cADPR[CH_2] (**143**) from their linear precursors.

Prior to this study it had been shown that cyclic ADP-ribose (cADPR) is a natural metabolite of NAD and a potent calcium-releasing second-messenger.[115] Substitution of the bridging pyrophosphate oxygen with methylene group resulted in compounds that are full agonist but with decreased agonist potency. These nucleotide analogues can be useful as a starting point for the development of membrane permeant cADPR prodrugs.[102]

Various kinases have been used in biocatalytic conversions of nucleoside diphosphates to the corresponding triphosphates.[30,51,116,117] In the example depicted in Scheme 58, synthesis of α,β-methylene-2′-deoxynucleoside 5′-triphosphates involves preparation of 2′-deoxynucleoside 5′-diphosphate precursors followed by an enzymatic γ-phosphorylation.[118] Enzymatic phosphorylation has been shown to be more efficient than the chemical approach for preparation of α,β-methylene-dNTPs. The α,β-methylene dNDP analogues examined are poor substrate for pyruvate kinase (PK). Therefore, the authors employed the substrate nonspecific nucleoside diphosphate kinase (NDPK). All synthesized α,β-methylene-dNTPs were found to be potent inhibitors of polymerase β, with K_i values ranging 1-5 μM.



Scheme 58. Synthesis of α,β-methylene-2´-deoxynucleoside 5´-triphosphates via a combination of chemical and enzymatic reactions.



144, **145**, and **146**: base = A
147: base = N^4-Bz-C
148, **149**: base = C

Scheme 59. Synthesis of α,β-difluoromethylene deoxynucleoside 5´-triphosphates utilizing a modified chemical-enzymological approach.

McKenna and co-workers have recently described the synthesis of α,β-difluoromethylene deoxynucleoside 5´-triphosphates (α,β-CF_2 dNTPs, N = A or C) using a modified chemical-enzymological approach.[16] They first converted dA or N^4-benzoyl-dC to the corresponding 5´-tosylates **144** or **147**, respectively, by reaction with tosyl chloride in pyridine. The tosylates were converted to the dNTP α,β-CF_2 analogues **145** or **148** via condensation with the tris(tetrabutylammonium) salt of difluoromethylenebisphosphonic acid. Phosphorylation to the dNTP analogues **146** or **149** was achieved using nucleoside diphosphate kinase and a catalytic amount of ATP, regenerated with 2.5 eq. of phosphoenolpyruvate (PEP) with pyruvate kinase (PK) in 50 mM HEPES buffer (Scheme 59). The latter modification renders unnecessary the use of an affinity column to purify the product from excess ATP.

3. Conclusion

In 2016, *gem*-bisphosphonates celebrated 45 years of application in medicinal chemistry, but in view of the burgeoning interest in bisphosphonate drugs and the concomitant desirability of expanding the structural scope of this class, it is not surprising that the synthesis of nucleoside polyphosphate analogues containing the P-CXY-P structural motif remains a challenging topic, and the development of highly efficient methodologies for synthesis of these species is of significant importance in biology and medicine. Landmarks in the development of contemporary chemistry of bisphosphonate analogues of nucleoside polyphosphates include Blackburn's synthesis of β,γ-fluoromethylene-bridged analogues of adenosine triphosphate and guanosine triphosphate (1984), Wang's synthesis of 2´,3´-dideoxynucleoside 5´-α-P-borano-β,γ-(difluoromethylene)-triphosphates (2005), Prakash's synthesis of fluorinated deoxynucleoside analogues based on bis(difluoro-methylene)triphosphoric acid (2010), Pankiewicz's synthesis of the mycophenolic adenine dinucleotide as a potent inhibitor of *h*IMPDH and leukemia K562 cells proliferation (2011), and McKenna's and Goodman's synthesis of the first individual β,γ-CHX-dGTP diastereomers [(*R*)- or (*S*)-CHX, where X is F or Cl] and determination their structures in ternary complexes with DNA polymerase β (2012). An area of great promise remains regio- and diastereoselective synthesis of nucleoside polyphosphate analogues with CXY-modified phosphate chains; much attention will be focused in the recent years to realize this problem. Moreover, in the next few years further studies to obtain details concerning the interactions of such species with enzymatic binding partners are likely to be the next challenge in bioorganic chemistry.

Acknowledgements

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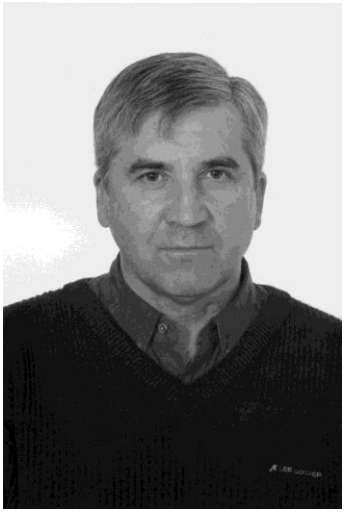
Authors Biographies



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