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## Synthetic strategies for non-hydrolyzable pyrophosphate analogues

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### Citation

Guo, J. (2026, September 17). *Synthetic strategies for non-hydrolyzable pyrophosphate analogues*. Retrieved from <https://hdl.handle.net/1887/4309697>

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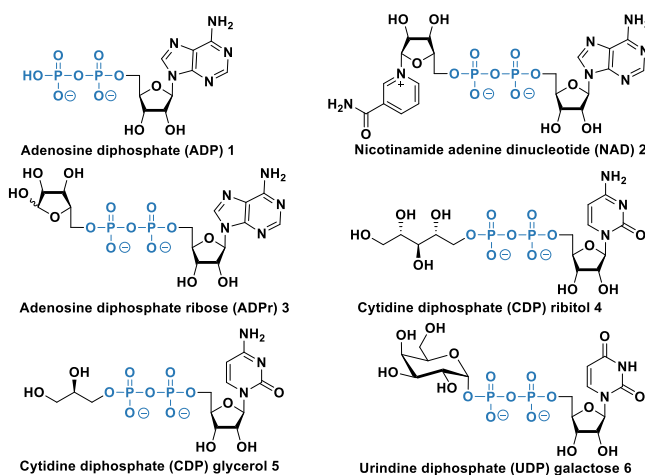
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# **Chapter 1**

## **General Introduction**

## 1.1 Introduction

Pyrophosphates are ubiquitous structural motifs in biomolecules, that play an all-important role in numerous biological processes.<sup>1-8</sup> As shown in Figure 1.1, adenosine diphosphate (ADP, **1**) is generated through the hydrolysis of adenosine triphosphate (ATP) and serves as a crucial molecule in cellular energy metabolism.<sup>9</sup> Nicotinamide adenine dinucleotide (NAD<sup>+</sup>, **2**) act as a cofactor in a plethora of enzyme-catalyzed oxidations in living systems.<sup>10</sup> NAD<sup>+</sup> is also used to install a post-translational modification (PTM) on proteins, referred to as adenosine diphosphate ribosylation. In this PTM, various protein nucleophilic side chains can be decorated with an adenosine diphosphate ribose (ADPr) moiety (as in **3**). These PTMs play a role in various cellular processes, including metabolism, signal transduction, and DNA repair.<sup>11-12</sup> Cytidine diphosphate (CDP) ribitol **4** and cytidine diphosphate (CDP) glycerol **5** are crucial building blocks for the biosynthesis of bacterial capsular polysaccharides and teichoic acids.<sup>13</sup> Nucleotide diphosphate (NDP) sugar donors, such as uridine diphosphate (UDP) galactose **6** are Nature's building blocks to construct complex carbohydrates and glycoconjugates.<sup>14</sup>

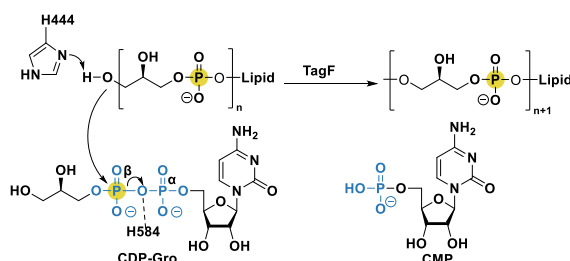


**Figure 1.1.** Examples of natural pyrophosphate molecules.

Despite the central roles of these pyrophosphate-containing molecules as substrates or cofactors in a wide range of essential metabolic and biosynthetic pathways, the rational development of inhibitors targeting these enzymes remains challenging. Structurally, a pyrophosphate group consists of two phosphate units connected through a bridging oxygen atom, forming a characteristic P-O-P bond. This moiety is labile, making it prone to hydrolytic and enzymatic cleavage. Non-hydrolyzable pyrophosphate analogues can be used as inhibitors for enzymes that process these substrates or as structural probes to investigate enzyme structure and function. This thesis focuses on the development of new non-hydrolyzable pyrophosphate analogues. Accordingly, novel reagents and synthetic strategies have been developed to access stabilized pyrophosphate bioisosteres for biochemical applications.

## 1.2 CDP-glycerol and CDP-ribitol in WTA Biosynthesis

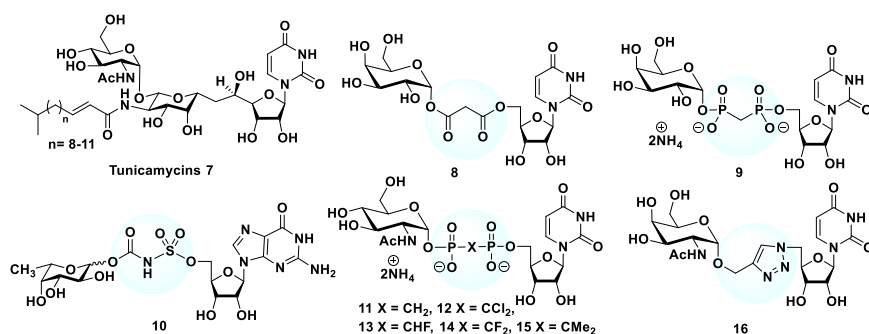
Wall teichoic acids (WTAs) are anionic cell wall polymers of Gram-positive bacteria that are involved in processes such as cell division, cell shape maintenance, and peptidoglycan synthesis.<sup>13</sup> Importantly, WTAs are key virulence factors, are linked to antibiotic resistance and have thus been widely studied as targets for therapeutic intervention.<sup>15-17</sup> The best-characterized WTA structures contain repeating units of ribitol 5-phosphate (RboP) or glycerol 3-phosphate (GroP). They are produced by important human pathogens such as *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Bacillus subtilis* and *Listeria monocytogenes*.<sup>18-20</sup> Poly(RboP) WTA biosynthesis has been characterized in both *B. subtilis* W23 and *S. aureus*, enzymes responsible for poly(RboP) WTAs assembly are designated Tar (teichoic acid ribitol). In contrast, poly(GroP) WTA biosynthesis has been characterized only in *B. subtilis* 168, where the corresponding enzymes are designated Tag (teichoic acid glycerol). WTA is sequentially synthesized by a series of enzymes on a lipid-linked precursor anchored to undecaprenyl phosphate (Und-P), containing the conserved linkage unit *N*-acetylmannosamine ( $\beta 1 \rightarrow 4$ ) *N*-acetylglucosamine 1-phosphate [ManNAc( $\beta 1 \rightarrow 4$ )GlcNAc-1P].<sup>21-22</sup> In both types of WTA biosynthesis pathways, TarB (TagB in *Bacillus subtilis* 168) catalyzes the addition of a glycerol phosphate residue to the linker disaccharide using CDP-glycerol as the glycerol phosphate donor.<sup>22-23</sup> After this step, WTA pathways diverge. In *S. aureus*, TarF mediates the transfer of a (predominantly) single unit of GroP from CDP-glycerol, after which TarL catalyzes the incorporation and subsequent polymerization of ribitol phosphate units using CDP-ribitol as donor to establish the characteristic RboP-type WTA backbone.<sup>20, 24-26</sup> In contrast, in *B. subtilis* W23 elongation of this RboP-type chain requires two enzymes: TarK, which transfers a single ribitol phosphate residue to the linkage unit, and TarL, which catalyzes the polymerization of the ribitol phosphate backbone, attaching more than 40 RboP units.<sup>25-26</sup> A comparable but distinct pathway operates for GroP-type WTA backbone in *B. subtilis* 168. After TagB completes the transfer of a glycerol phosphate residue to the linker disaccharide, TagF functions as the glycerol phosphate polymerase, adding 45–60 GroP units to the TagB product using CDP-glycerol as a donor.<sup>27-29</sup> In this TagF-catalysed reaction, TagF binds CDP-glycerol and lipid to form a ternary complex. H444, as an active-site base, deprotonates the terminal hydroxyl group of the polymer, generating a nucleophile that attacks the  $\beta$ -phosphate of CDP-glycerol. The ternary complex subsequently dissociates to liberate free enzyme, CMP, and the elongated GroP-type chain (Figure 1.2).<sup>30-31</sup> In *S. aureus*, a similar catalytic mechanism has been proposed for the WTA polymerase TarL, which uses CDP-ribitol as the substrate for the elongation of RboP-type WTA chains. Due to their critical role in pathogenesis, understanding WTA biosynthetic enzymes provides insights for the development of novel inhibitors and potential therapeutic agents.



**Figure 1.2.** Proposed mechanism for glycerol-phosphate polymerization catalyzed by TagF in *Bacillus subtilis* 168.

### 1.3 Rational design of stable pyrophosphate analogues for glycosyltransferase inhibition

Glycosyltransferases (GTs) are a large family of enzymes responsible for the construction of glycosidic linkages through the transfer of a sugar moiety from activated sugar donors (most commonly NDP-sugars) to acceptors.<sup>32-33</sup> The negatively charged pyrophosphate group of the NDP-sugar donor is typically engaged either by coordinating with divalent metal ions or by interacting with positively charged amino acid side chains, depending on the structural fold. These interactions help stabilize the pyrophosphate of the donor and properly position it within the active site, thereby facilitating substrate binding, and additionally promoting catalysis in certain GTs. Disruption of such interactions may diminish binding affinity. Nevertheless, natural glycosyltransferase inhibitors such as tunicamycin (**7**, Figure 1.3), which lack a pyrophosphate unit, indicate that alternative structural motifs can effectively mimic these key interactions. This observation provides a rationale for the design of non-hydrolyzable nucleotide-sugar analogues as chemical tools and potential selective GT inhibitors. Deregulation of GTs expression or activity can lead to abnormal glycosylation and is associated with the onset of various diseases. Rationally designed non-hydrolyzable nucleotide-sugar analogues have therefore emerged as powerful chemical tools to investigate GT activity, selectivity, and catalytic mechanisms, and also hold potential for therapeutic intervention through selective enzyme inhibition. For example, analogues **8** and **9** (Figure 1.3),<sup>34, 35</sup> in which the phosphate group was substituted with malonate and methylene bisphosphonate, respectively, were designed as potential inhibitors of galactosyltransferases. Analogue **10** featuring an oxysulfonyl carbamate was developed as an inhibitor of fucosyltransferase.<sup>36</sup> In analogues **11-15**, the bridging oxygen atom of the pyrophosphate moiety was replaced by a methylene (CH<sub>2</sub>), dichloromethylene (CCl<sub>2</sub>), fluoromethylene (CHF), difluoromethylene (CF<sub>2</sub>), or *iso*-propylene (CMe<sub>2</sub>) group, respectively. These analogues were designed to target phosphoglycosyl transferases.<sup>37</sup> Methylene-triazole linked analogue **16** was designed as an inhibitor of *N*-acetylgalactosaminyltransferases.<sup>38</sup> Besides glycosyltransferases, rationally designed stable pyrophosphate analogues have also been successfully applied to inhibit a variety of other enzyme classes that utilize nucleotide diphosphate as a substrate.<sup>39-41</sup>



**Figure 1.3.** Chemical structures of tunicamycin and non-hydrolyzable nucleotide-sugar analogues.

### 1.4 Stabilized pyrophosphate analogues

Pyrophosphate groups are intrinsically predisposed to hydrolysis, transesterification, and enzymatic cleavage.<sup>42-43</sup> As a result, natural pyrophosphate-containing molecules are generally unstable under biological conditions, complicating the study of their functions. Non-hydrolyzable analogues of these natural molecules serve as useful chemical tools for structural studies and inhibitor development. Over the years, a variety of chemical structures have been explored to closely mimic the pyrophosphate moiety (Figure 1.3 and 1.4), including oxysulfonyl carbamate,<sup>36</sup> bisphosphonates (BPs),<sup>44</sup> imidodiphosphates,<sup>45-48</sup> thiopyrophosphate,<sup>39</sup> selenophosphates,<sup>49-50</sup> boranophosphates,<sup>51</sup> phosphonoacetates<sup>40</sup> and malonate moiety.<sup>34</sup> Although these analogues differ in structure, each class of mimics preserves some key features of the native pyrophosphate and offers distinct advantages in terms of stability and resistance to hydrolysis. Among these, imidodiphosphates closely mimic the bond lengths and angles of natural pyrophosphate and may enhance binding through additional hydrogen-bonding interactions.<sup>45-48</sup> Substitution of oxygen with isoelectronic sulfur or selenium gives rise to thiophosphate and selenophosphate analogues with increased hydrolytic stability. Similarly, boranophosphates, in which a nonbridging oxygen is replaced by a borane (BH<sub>3</sub>) group, preserve the negative charge of the phosphate while enhancing lipophilicity and nuclease resistance.<sup>51</sup> A phosphonoacetate motif has also been utilized as a stable pyrophosphate mimic, since the P–C bond is more chemically stable than the pyrophosphate P–O bond. In addition, malonate was introduced as a bioisosteric replacement for the pyrophosphate group to mimic the pyrophosphate–Mn<sup>2+</sup> coordination complex.<sup>34</sup> Among the various strategies for pyrophosphate mimicry, replacement of the bridging oxygen atom with methylene- or difluoromethylene (P–CH<sub>2</sub>–P and P–CF<sub>2</sub>–P) linkages have attracted considerable attention.

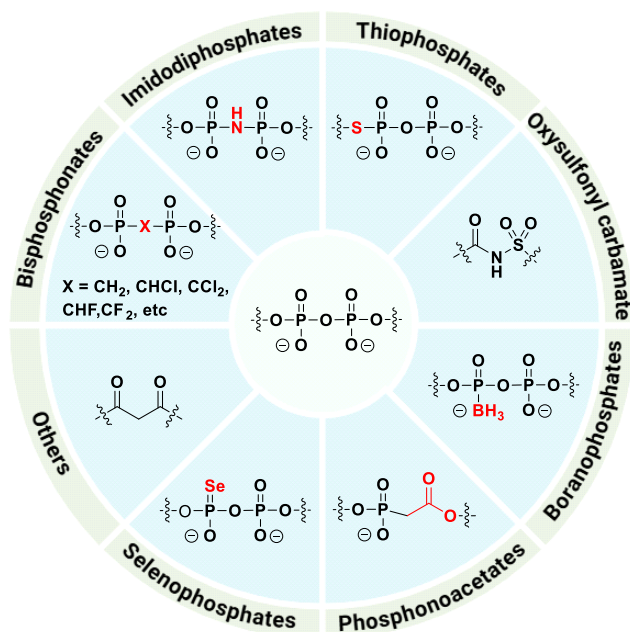
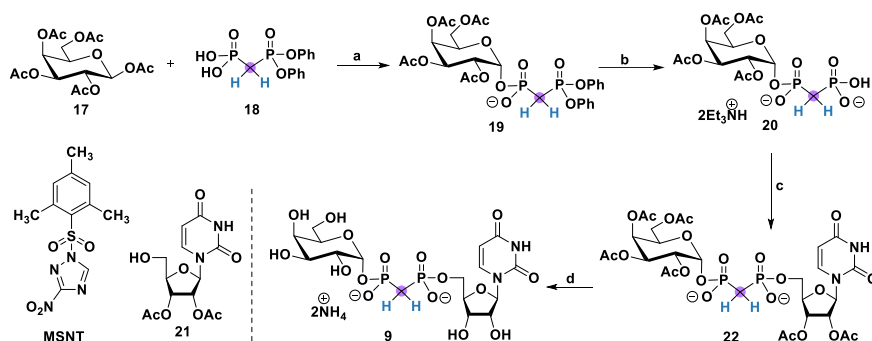


Figure 1.4. Stable chemical mimetics of the pyrophosphate moiety.

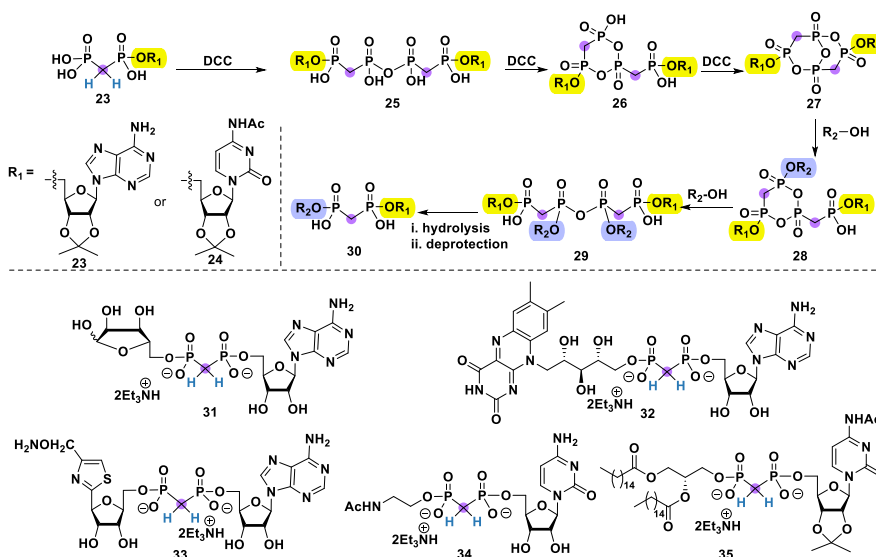
#### 1.4.1 Methylene bisphosphonate (P–CH<sub>2</sub>–P) diesters as pyrophosphate analogues

Methylene bisphosphonates (P–CH<sub>2</sub>–P) represent an important class of pyrophosphate bioisosteres, in which the central oxygen atom of the native pyrophosphate linkage is replaced by a methylene group. This substitution introduces minimal structural changes while rendering the linkage resistant to enzymatic and chemical cleavage. These analogues have been used to elucidate the functional roles of a variety of enzymes.<sup>37, 52-53</sup> Building on their biological utility, several synthetic strategies have been established for their construction. For instance, Vaghefi *et al.* reported the synthesis of a P–CH<sub>2</sub>–P linked UDP–Gal analogue (Scheme 1.1).<sup>35</sup> β–D–galactose pentaacetate was employed to couple with diphenyl phosphonic acid at high temperature (170 °C) to yield diphenyl phosphonate **19** in 46% yield, followed by hydrogenation over platinum oxide to afford **20**. This intermediate was subsequently condensed with 2,3–diacetyluridine **21** in the presence of 1–(mesitylene–2–sulfonyl)–3–nitro–1,2,4–triazole (MNST) to provide the protected UDP–Gal analogue **22**. Final deacetylation, followed by purification using an anion–exchange resin, yielded the target analogue **9**.



**Scheme 1.1.** Synthesis P-CH<sub>2</sub>-P linked UDP-Gal analogue **9**. Reagents and conditions: a) vacuum, 170 °C, 46%. b) Pt<sub>2</sub>O, H<sub>2</sub> (1000psi), 40 °C, overnight, 52%. c) **21**, pyridine, MSNT, rt, overnight. d) NH<sub>4</sub>OH, rt, 3 h, 38% over two steps.

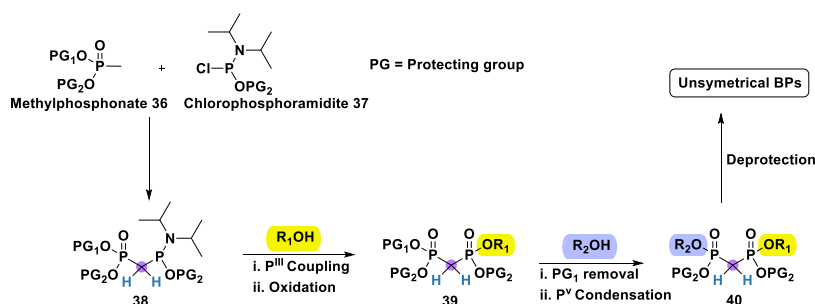
Another approach is via carbodiimide activation, as reported by Pankiewicz *et al* (Scheme 1.2).<sup>54</sup> Treatment of P-CH<sub>2</sub>-P linked ADP **23** with stoichiometric DCC in pyridine results in rapid dimerization to give the dimer **25**, which was isolated but found to be unreactive toward alcohols. Intermediate **25** was further activated through two successive DCC-mediated condensations to afford the highly reactive nucleoside bicyclic trisanhydride **27**. The reaction of **27** with an alcohol at 60 °C enabled nucleophilic attack at either bisphosphonic position to form intermediate



**Scheme 1.2.** Synthesis of P-CH<sub>2</sub>-P linked analogues via carbodiimide activation. Analogues **31–33** were prepared from compound **23** by reaction with 2,3-*O*-isopropyl D-ribose, riboflavin, and 2,3-*O*-isopropyl thiazofurin, respectively, followed by hydrolysis and deprotection. Analogues **34** and **35** were obtained from compound **24** by reaction with *N*-acetyethanolamine and 1,2-dipalmitoyl-*sn*-glycerol, respectively, under the same coupling, hydrolysis and deprotection conditions.

**28**, which was subsequently attacked by a second molecule of alcohol to provide dimer **29**. This was followed by hydrolysis and deprotection to afford unsymmetric methylene bisphosphonate **30**. Based on this methodology, a series of ADPr, flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), CDP-*N*-acetyethanolamine, and CDP-diacylglycerol (CDP-DAG) methylene bisphosphonate analogues (**31–35**) were synthesized.

Other methods for the synthesis of bisphosphonate analogues have also been reported, including nucleophilic displacement of good leaving groups (such as OTs and OMs),<sup>55</sup> and Mitsunobu esterification.<sup>56</sup> Although these methods provided valuable access to P-CH<sub>2</sub>-P analogues, they often suffer from harsh conditions and restricted control over the substitution pattern at the bisphosphonate core. To overcome these limitations and to enable a more modular construction of unsymmetric analogues, Engelsma *et al.* developed a versatile protocol for the synthesis of P-CH<sub>2</sub>-P analogues (Scheme 1.3).<sup>57</sup> A new orthogonally protected phosphonylmethylphosphonamidite reagent **38** was generated by treating lithiated methylphosphonate diesters **36** with chlorophosphoramidite **37**. This reagent can undergo efficient coupling with a variety of alcohols via a P<sup>III</sup> phosphoramidite coupling/oxidation sequence to give compound **39**. Selective deprotection of the PG<sub>1</sub> group and reaction with a second alcohol through a P<sup>V</sup> condensation provides precursors for unsymmetric bisphosphonates. This strategy has enabled the efficient incorporation of methylene bisphosphonate into diverse pyrophosphate-containing molecules, such as ATP, ADPr and FAD. Because of its effectiveness in the condensation of secondary alcohols, this method was applied in the synthesis of P-CH<sub>2</sub>-P linked NDP-sugar analogues as described in **Chapter 3** and **Chapter 5**.

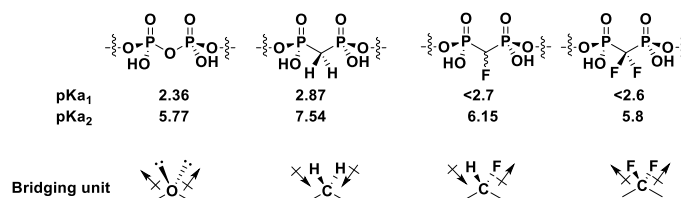


**Scheme 1.3.** Unsymmetric methylene bisphosphates synthesis via a phosphonylmethylphosphonate reagent.

#### 1.4.2 Difluoromethylene bisphosphonate (P-CF<sub>2</sub>-P) analogues

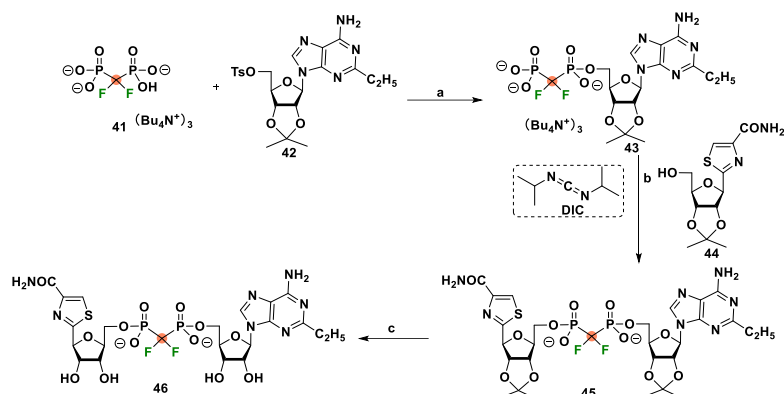
The difluoromethylene bisphosphonate (P-CF<sub>2</sub>-P) analogues are of particular interest, since they most closely mimic the natural pyrophosphates.<sup>58–62</sup> This close resemblance arises from several key features. First, the electronegativity of the fluorine atoms affords the difluoromethylene bisphosphonic acid a pK<sub>a</sub> value (pK<sub>a</sub> <2.6) close to pyrophosphoric acid (pK<sub>a</sub> 2.36), and thus results in a similar protonation state to that of native pyrophosphate under physiological conditions. Second, the overall geometry of the P-CF<sub>2</sub>-P moiety closely resembles that of the natural P-O-P linkage in terms of bond lengths, bond angles, and “right polarity” (Figure 1.5).

This structural similarity allows the P–CF<sub>2</sub>–P–linked analogue to occupy the same binding space as the parent structure within the enzyme active site, minimizing steric perturbation while preserving essential molecular interactions. The polar nature of the CF<sub>2</sub> group also supports hydrogen bonding and electrostatic interactions within the binding pocket. All these properties make P–CF<sub>2</sub>–P analogues attractive candidates for use as stable chemical tools and inhibitors.



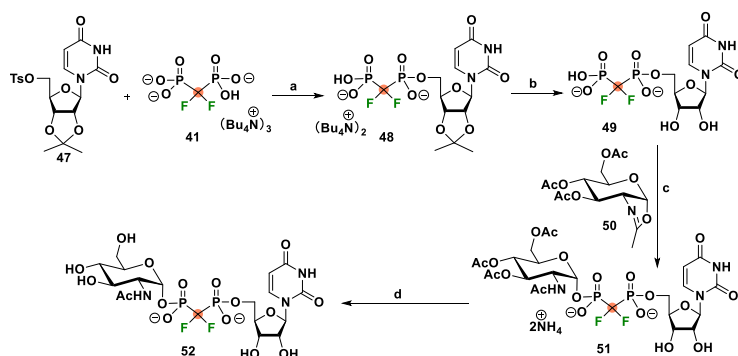
**Figure 1.5.** pKa Values and polarity of pyrophosphoric acid and its analogues.

The synthesis of unsymmetric difluoromethylene bisphosphonate diesters is challenging because the strong electron-withdrawing effect of the difluoromethylene group significantly reduces the nucleophilicity of the phosphonate anions. Furthermore, the selective installation of different substituents on either side of the difluoromethylene bisphosphonate remains difficult. There are only a limited number of examples in literature, the first step in almost all cases is achieved by reacting tris(tetra-*n*-butylammonium) salts of difluoromethylene bisphosphonate **41** with a primary tosylate, such as 5-tosyl nucleoside **42**, to afford a bisphosphonate intermediate such as **43** (Scheme 1.4). This approach was first reported in 1979 by Stock who used the trialkylammonium salt of methylenebisphosphonic acid and reacted this with 5-tosyl-thymidine to yield the corresponding P–CH<sub>2</sub>–P analogues.<sup>63</sup> Later, this approach was adapted by Poulter and co-workers to synthesize a variety of P–CF<sub>2</sub>–P analogues.<sup>64</sup> Building on this strategy, Felczak and co-workers designed and synthesized analogues that mimic nicotinamide adenine dinucleotide (NAD), in which the pyrophosphate was replaced by a difluoromethylene bisphosphonate linkage, while the nicotinamide unit was replaced with a thiazole-4-carboxamide group (Scheme 1.4).<sup>65</sup> After obtaining the intermediate **43**, the coupling on the opposite side was achieved using DIC or similar dehydrating agent to mediate the condensation reaction to produce unsymmetric difluoromethylene bisphosphonate analogue **45**. Subsequent deprotection provided the analogue **46**, which acted as a selective inhibitor of inosine monophosphate dehydrogenases (IMPDH1 and IMPDH2), displaying potent inhibition of human IMPDH1, with a K<sub>i</sub> of approximately 5 nM.



**Scheme 1.4.** Synthesis of P-CF<sub>2</sub>-P linked thiazole-4-carboxamide adenine dinucleotide. Reagents and conditions: a) MeCN, rt, 24 h, 72%. b) (i) DIC, pyridine, rt, overnight; (ii) **44**, 62 °C, 70 h; (iii) H<sub>2</sub>O/Et<sub>3</sub>N (9:1 v/v), overnight, rt. c) 50% HCOOH, overnight, rt, 20% over 4 steps.

Another example of an unsymmetric difluoromethylene bisphosphonate analogue was presented by Imperiali and coworkers (Scheme 1.5).<sup>37</sup> The first steps of their approach followed the same strategy as described above, and intermediate **48** was prepared via nucleophilic displacement of 5-tosyl-uridine **47** by bisphosphonate **41**. The 2',3'-isopropylidene group on compound **48** was then removed under acidic conditions and the resulting product was converted to the free acid **49**. The coupling on the opposite side of compound **49** was achieved using oxazoline **50** under glycosylation conditions to afford, after three days, the protected UDP-GlcNAc analogue **51** in 49% yield. Finally, global deprotection using basic conditions yielded the target P-CF<sub>2</sub>-P linked UDP-GlcNAc **52**. However, this methodology is limited to synthesizing specific substrates because it depends on oxazoline-mediated glycosylation, which restricts its use with structurally diverse pyrophosphate-containing molecules. Moreover, this method requires prolonged reaction times to achieve acceptable yields and necessitates RP-HPLC purification at each step.



**Scheme 1.5.** Synthesis of P-CF<sub>2</sub>-P linked UDP-Glc. Reagent and conditions: a) MeCN, rt, 48 h, 75%. b) aq. HCl (pH ~0.5), rt, 2 h, quantitative. c) (i) Dowex H<sup>+</sup>, H<sub>2</sub>O; (ii) GlcNAc oxazoline **50**, DMF, 45 °C, 3 days, 49%. d) aq. NH<sub>4</sub>OH (pH 10.5-11.5), rt, 2.5 h, quantitative. Yields were defined by UV.

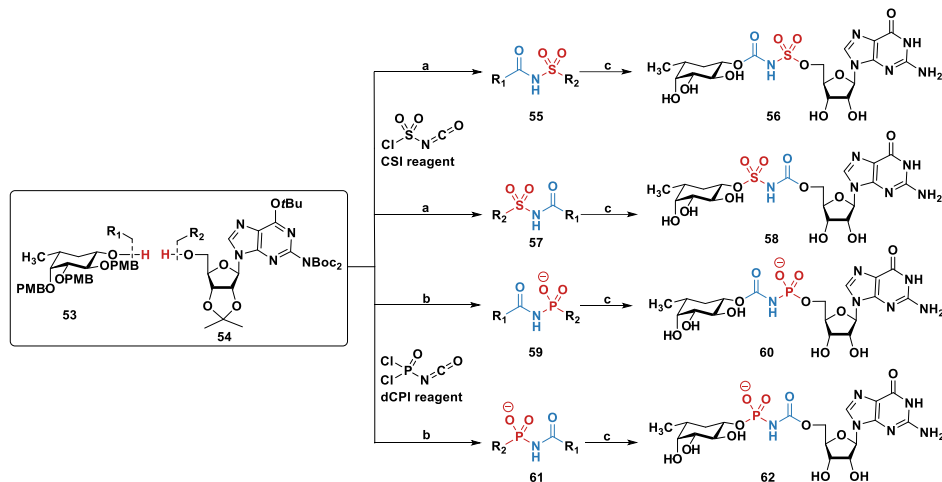
Although carbodiimide-mediated coupling and nucleophilic displacement of *O*-sulfonyl groups provide feasible routes for the synthesis of unsymmetrical difluoromethylene bisphosphonate analogues, only a few examples have been reported to date. Moreover, these reactions require the installation of leaving groups on the substrates, which can lead to side reactions. The generation of charged intermediates, makes purification challenging. Therefore, the development of more general and efficient methods for accessing unsymmetrical difluoromethylene bisphosphonate analogues is desirable to broaden their applications in chemical biology and enable the generation of new inhibitors. This is described in detail in **Chapter 4**.

The coupling of primary alcohol with difluoromethylene bisphosphonate is limited by the lack of general synthetic methods and this holds true even more for the coupling of a secondary alcohol. Although Imperiali's method provides a route to P-CF<sub>2</sub>-P UDP-GlcNAc analogue, the resulting glycosidic bond remains susceptible to enzymatic cleavage. Carbasugars are widely used in studies of carbohydrate-processing enzymes and offer a more stable alternative.<sup>66-70</sup> Therefore, the synthetic strategy described in **Chapter 4** was applied and further optimized to access a P-CF<sub>2</sub>-P linked UDP-carbaGal analogue with both the glycosidic bond and the pyrophosphate linkage being replaced by non-hydrolyzable moieties as described in **Chapter 5**.

### 1.4.3 Oxsulfonyl carbamate analogues

In addition to analogues that substitute one or more oxygen atoms of the pyrophosphate or replace the bridging oxygen, other strategies have introduced structurally distinct units in place of the pyrophosphate moiety. Camarasa *et al.* showed the benefits of using chlorosulfonyl isocyanate (CSI) as a reagent to assemble uridine-diphosphate glucose (UDP-Glc) mimic in which the pyrophosphate linkage was replaced by an oxsulfonyl carbamate.<sup>71</sup> This reagent enabled the rapid introduction of carbonyl and sulfonyl groups as bioisosteric replacements for the natural pyrophosphate moiety, as demonstrated in several reported examples.<sup>36, 72</sup> More recently, Steneker *et al.* extended phosphoryl carbamate linkages as alternative bridging unit using dichlorophosphoryl isocyanate (dCPI) reagent, and developed a modular coupling strategy to construct a series of NDP-sugar analogues featuring either sulfonyl carbamate or phosphoryl carbamate linkages (Scheme 1.6).<sup>73</sup> To construct these derivatives, carba- $\beta$ -fucose **53** was employed, in which the ring oxygen is replaced by a methylene group to enhance its stability. Following the reported procedure, alcohol **53** was first treated with CSI at low temperature to yield carbamate sulfonyl chloride intermediate, which was directly coupled with guanosine **54** to give the fully protected GDP-fucose analogue **55**, followed by deprotection to yield the analogue **56**. Reversing the order of addition of the two nucleophiles, followed by acid deprotection, enabled the successful synthesis of regioisomer **58**. Following a similar procedure, carba-sugar **53** was treated with dCPI at 0 °C, followed by nucleophilic chloride displacement with **54** in the presence of pyridine. Subsequent hydrolysis of the resulting chlorophosphoryl carbamate intermediate under aqueous conditions afforded the protected analogue **59**. Regioisomer **61** was obtained by reversing the order of the nucleophiles. Subsequently, acidic deprotection afforded compounds **60** and **62**. This methodology provides a flexible approach for the installation of

sulfonyl carbamate or phosphoryl carbamate linkages to replace the native pyrophosphate moiety, and was applied in this thesis to prepare a series of CDP-Gro and CDP-Rbo mimics. This is described in detail in **Chapter 2**.

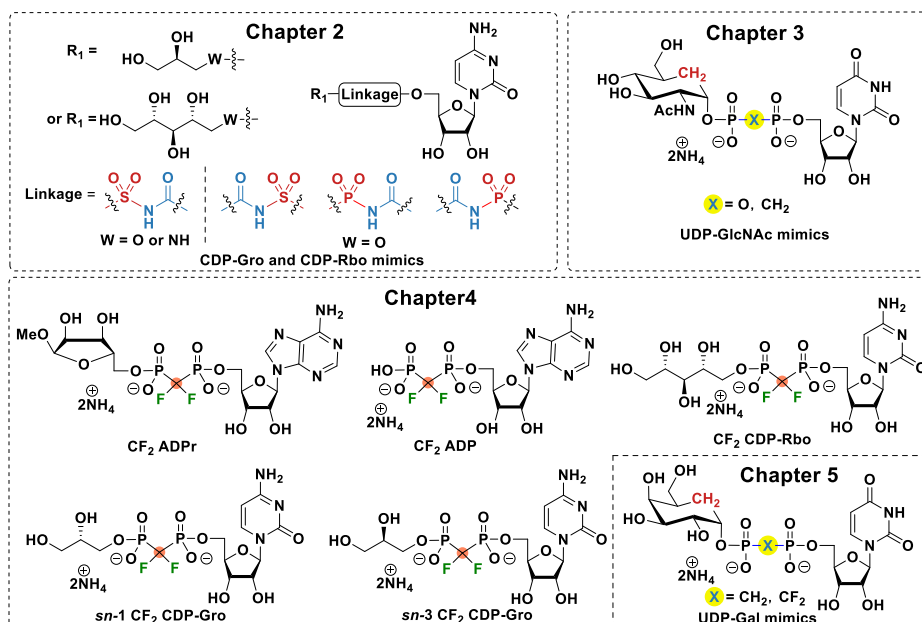


**Scheme 1.6.** Synthesis of GDP-fucose analogues featuring sulfonyl carbamate or phosphoryl carbamate linkages. Reagents and conditions: a) (i) CSI (1.0 equiv), nucleophile **1** (1.0 equiv), 3 Å MS,  $\text{CH}_2\text{Cl}_2$ , 0 °C; (ii) nucleophile **2** (1.0 equiv), pyridine (3.0 equiv),  $\text{CH}_2\text{Cl}_2$ , 0 °C to rt, **55**, 42%; **57**, 54%. b) (i) dCPI (1.0 equiv), nucleophile **1** (1.0 equiv), 3 Å MS,  $\text{CH}_2\text{Cl}_2$ , 0 °C; (ii) nucleophile **2** (1.0 equiv), pyridine (6.0 equiv),  $\text{CH}_2\text{Cl}_2$ , 0 °C to rt; (iii)  $\text{H}_2\text{O}$ ; c) TFA (30% v/v),  $\text{CH}_2\text{Cl}_2$ , **56**, 69%; **58**, 45%; **60**, 5% over two steps, **62**, 14% over two steps.

## 1.5. Content of this thesis

The research described in this Thesis aims to develop stable pyrophosphate mimics. New reagents and methodologies are developed and existing methods are optimized to facilitate these studies. **Chapter 2** describes the design and synthesis of stabilized CDP-glycerol and CDP-ribitol analogues incorporating *O*- or *N*-sulfonyl carbamate and phosphoryl carbamate groups, as potential inhibitors of wall teichoic acid (WTA) biosynthesis enzymes (Figure 1.6). **Chapter 3** describes the preparation of two UDP-GlcNAc analogues as glycosyltransferase inhibitors using a phosphoramidite coupling strategy and a newly developed orthogonally protected phosphonylmethylphosphonate reagent. The reagent and the complementary methodology were further employed in **Chapter 5**. **Chapter 4** describes an efficient orthogonal deprotection–condensation strategy enabling a stepwise functionalization sequence for the coupling of primary alcohols with a symmetrical difluoromethylene bisphosphonate reagent. The key reagent could be generated on a large scale, providing sufficient quantities for the preparation of various unsymmetrical difluoromethylene bisphosphonate diesters. The versatility of this strategy is demonstrated in the construction of a series of mimics of natural pyrophosphate molecules. The newly developed methodology is further applied to the synthesis of UDP-galactose analogues in

**Chapter 5.** The first part of **Chapter 5** focuses on the synthesis of carbagalactose, the building block that is required for the formation of UDP-galactose analogues. The second part further employs the reagents synthesized in **Chapter 3** to prepare P-CH<sub>2</sub>-P UDP-carbagalactose. In addition, further optimization of the methodology described in **Chapter 4** enabled its extension to the coupling of secondary alcohols, thereby allowing the synthesis of P-CF<sub>2</sub>-P UDP-carbagalactose. Furthermore, the inhibitory activities of these analogues against A4GALT were evaluated. **Chapter 6** summarizes the research described in this thesis and suggests future research directions based on the design of mimics of pyrophosphate-containing molecules and on the methodologies developed throughout this work.



**Figure 1.6.** Overview of the analogues described in this thesis.

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